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A LABOR-SAVING DEVICE FOR THE COLLECTION OF NEMATODES[1]

A. C. Tarjan, W. A. Simanton, and E. E. Russell

SUMMARY

A washing apparatus for the extraction of nematodes from soil and from comminuted plant tissue has been devised. It uses spray nozzles for the mechanical washing of the sample and a system of inclined, graded sieves for catching the nematodes. Results of 3 experiments indicated that approximately 96 per cent of the nematodes in a given sample can be obtained by washing for 10 minutes.

One of the basic requirements in many nematological investigations is utilization of techniques that will enable workers to make qualitative and perhaps quantitative determinations of the nematodes in a given sample. To this end, Stocki (9, 10) and Overgaard (8) have suggested methods for working with soil-inhabiting nematodes. These methods mainly employ the principle suggested by Baermann (3). A modified Baermann funnel method was reported by Anderson and Yanagihara (2), who used paraffin-coated paper cups in place of the usual glass funnel that accommodates the nematode-infested sample. Another novel technique has been reported by Caveness and Jensen (4), who employed a centrifugal-flotation principle and a sugar-water syrup for obtaining large quantities of nematodes and their eggs. None of such devices, however, permitted relatively rapid or efficient screening of larger volumes of soil. Oostenbrink (7) proposed a flotation washer that utilized innovations by Ahlberg (1) and Fenwick (6). The intrinsic value of Oostenbrink's apparatus is that it greatly reduces the amount of labor necessary for the proper washing of soil samples.

This paper describes a washer fundamentally designed after Oostenbrink's apparatus but incorporating several modifications that enhance its workability. The new design includes 1) a system of spray jets for washing and agitating the soil, 2) a glass tube that enables one to observe the soil-washing operation, 3) a drain outlet with a jet nozzle at the bottom of the washing tube that permits cleaning out the tube without dismantling the apparatus, and 4) a system of inclined sieves that allows for a selective trapping of different sized nematodes and reduces the percentage loss of smaller nematodes through the fine screens.

DESCRIPTION AND OPERATION OF APPARATUS.—The entire apparatus, shown in operation in Figure 1, A, is mounted on a marine plywood backboard (18 × 48 in.) attached to a wheeled cart (24 × 24 × 12 in.). The component parts of the washing column, beginning at the top of the unit, may be described as follows. The water inlet tube (A) terminates in a spray-head assembly consisting of 3 whirljet type spray nozzles equally spaced in a triangular pattern. The removable spray shield (B) is comprised of a copper cylinder resting inside of sieve (C), which is an 8-in. diameter brass U.S. Standard Sieve of 10 mesh. The copper funnel (D) is 8½ in. in diameter and is fitted with a 10-in. stem made of ½-in. copper tubing. This funnel rests on a supporting bracket and in turn supports the sieve and the spray shield. The stem extends down about 8 in. into a glass tube whose upper end is fitted snugly with a neoprene collar (E) formed in the shape of a trough to divert the overflow water into the sieves. The glass tube (F) has an inside diameter of 2½ in., is 2 ft. long, and is supported by brackets extending from the backboard. A graded series of 4 standard sieves (G), ranging from medium to fine mesh, is used for trapping the nematodes. These sieves are supported by adjustable spring clips that permit the sieves to be readily removed for emptying. A water inlet and drainage assembly (H) is fitted into a No. 13 rubber stopper that serves to plug the lower end of the glass tube. The rubber stopper, as well as the glass tube, is supported by a slotted bracket extending out from the backboard. The drain tube (I) consists of ¾-in. (inner diameter) rubber tubing equipped with a large pinch clamp. Pressure tubing (J) is used to conduct water from the faucet to the lower spray nozzle assembly. An 8½-in. copper funnel equipped with a short stem and a length of tubing (K) receives the water that passes through the collecting sieves and conducts it to the drain. Details of the lower nozzle and drain assembly are shown in Figure 1, B.

Straight nozzles of the whirljet type for the lower nozzle assembly were obtained from the Speed Sprayer Factory, 1312 W. Washington Street, Orlando, Florida. Similar nozzles are sold by Spraying Systems Company, 3201 Randolph Street, Bellwood, Illinois. Orifice diameters of 0.025 to 0.05 in. usually provide the correct flow, depending on water pressure employed.

In preparation for washing nematodes from a soil sample, the pinch clamp on drainage tube (I) is closed. Water is then permitted to flow through the lower nozzle assembly at the rate of about 1 quart per minute. When the glass tube is overflowing slightly, the soil sample is placed in sieve (C) at the top of the unit. Water is then sprayed over the sample through the nozzle assembly (A), also at the rate of about 1 quart per minute. As the soil and water are introduced into the glass tube from above through funnel (D), the heavier soil particles sink to the bottom of the tube where they continue to be agitated by

[1] Accepted for publication June 29, 1956.
Florida Agricultural Experiment Station Journal Series, No. 489.

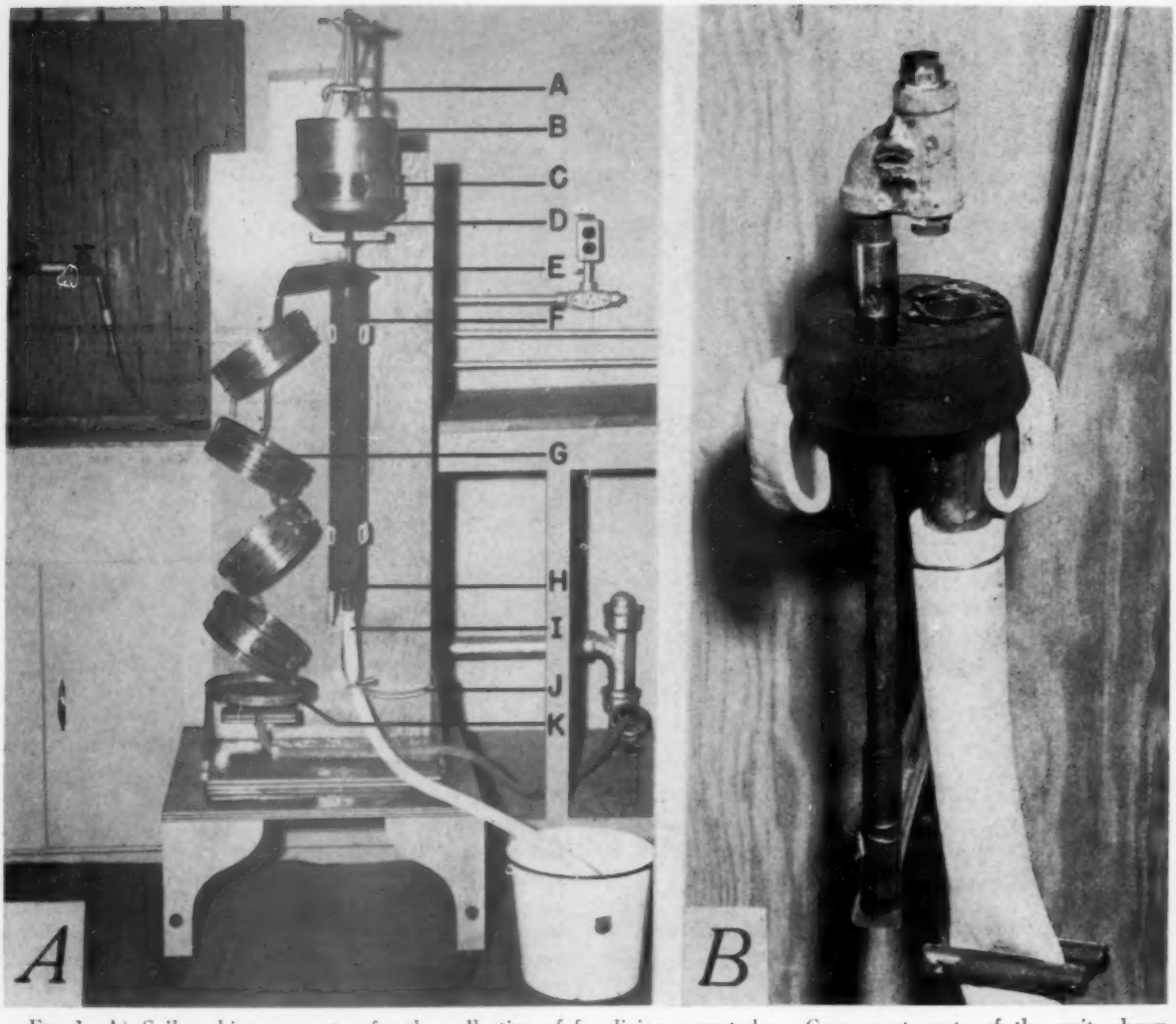

FIG. 1. A) Soil-washing apparatus for the collection of free-living nematodes. Component parts of the unit, shown here in operation, are described in the text. B) Construction of the lower tube-assembly showing spray nozzles that agitate the soil and facilitate cleaning of tube.

the lower spray assembly. The lighter soil particles, debris, and nematodes gradually move upward through the glass tube and flow over the top edge into the rubber lip, thence down through the graded series of sieves where the nematodes are trapped. The water passing through the sieves is drained off at the bottom.

After any specified washing period, the pinch clamp on the drain tube (I) is opened. Water from both upper and lower nozzles thereupon flushes the remaining soil down through the drainage tube (I) into a pail for subsequent disposal. The nematode-containing residues on the sieves are then collected and the cleaned sieves replaced. After sieve (C), funnel (D), and collar (E) are rinsed, the apparatus is ready to receive another sample.

For general use in collecting nematodes from soil samples, it has been found best to use a 60-mesh, a 200-mesh, and two 325-mesh U. S. Standard sieves set at opposing angles directly beneath each other (Fig. 1, A). Inclination of the sieves permits more effective retention of nematodes on the wire cloth; a stream of water meeting the wire cloth at a more perpendicular angle would have a tendency to force the nematodes through the mesh openings.

If the residues on the inclined sieves are combined in a beaker and a small amount of water added, a direct examination for nematodes can be made. In instances where fine soil particles also have been trapped on the screens, one can facilitate examination by decanting the combined water-nematode-debris mixture into another vessel, leaving behind the heavier particles. To obtain a clear suspension of nematodes in water, the screened residues can be transferred to a Baermann funnel as suggested by Christie and Perry (5).

The apparatus also has been used for collecting nematodes from infested plant material. This has been accomplished by comminuting the roots in a Waring

Blendor (11) and then introducing the root-water mixture directly to the 10-mesh sieve beneath the top spray assembly.

TESTS ON THE EFFICIENCY OF THE SOIL-WASHING APPARATUS.—Oostenbrink (7) found that his soil-washing apparatus was from 80 to 90 per cent efficient in "favorable" soils and that this efficiency might decrease in peat and heavy clay soils. Accordingly, the efficiency of the apparatus described in the present paper was tested in a series of experiments in which the number of nematodes collected on the sieves was compared with the number of nematodes remaining in the glass tube after various periods of washing.

In one experiment designed to determine the most effective size of aperture for the spray nozzles and the relative position of the lower spray assembly within the glass tube, it was found that lowering this spray assembly so that the bottom spray jet was located about 1 in. above the rubber stopper improved the efficiency of the washing process about 15 per cent. Selective testing of various sized spray nozzles resulted in the selection of a size that permitted the most efficient washing of the soil sample.

In 2 separate tests, each using a different soil sample containing roots of St. Augustine grass, duplicate pint samples were washed for 5, 10, 15 and 20 minutes. The sieved residues (referred to as whole samples for convenience) then were combined and put in Baermann funnels for quantitative determinations of nematodes. The washed soil in the glass tube remaining from these same samples was drained and then passed through a 60-mesh, a 200-mesh, and two 325-mesh standard sieves. The residues thus collected (referred to as tube samples) were combined and put in Baermann funnels. After 24 hours, 100 ml from each funnel was drawn out, the nematodes present in replicate 2-ml aliquots were counted, and the average count amplified to represent the approximate number in the 100-ml sample. Previous tests had indicated that the 100-ml samples contained the majority of the nematodes present in the samples placed in the respective Baermann funnels.

TABLE 1.—*Percentage recovery of nematodes from grass soil subjected to washing in the apparatus for various periods*

Test	Washing time in minutes	Average no. of nematodes in sample from indicated source		Percentage Recovery
		Whole[a]	Tube[b]	
1	5	1450	50	97
	10	750	50	93
	15	500	0	100
	20	700	50	93
2	5	3250	175	95
	10	1875	75	96
	15	3250	125	96
	20	1850	100	95

[a] Combined sieved residues.
[b] Washed soil remaining in glass tube.

TABLE 2.—*Percentage recovery of nematodes from 3 different soils subjected to washing for various periods*

Soil type	Washing time in minutes	Average no.[a] of nematodes in sample from indicated source		Percentage recovery
		Whole[b]	Tube[c]	
Muck	5	35	3	91
	10	83	7	92
	15	190	0	100
	20	310	0	100
Grass	5	1917	33	98
	10	1333	33	98
	15	1333	67	95
	20	917	50	95
Citrus	5	150	17	89
	10	283	0	100
	15	117	0	100
	20	250	0	100

[a] Figures are averages of 3 replicate pint samples of soil, with 2 nematode counts obtained from each.
[b] "Whole" refers to combined sieved residues.
[c] "Tube" refers to washed soil that remained in the glass tube.

Over 90 per cent of the nematodes in each pint sample were recovered on the sieves regardless of the duration of washing (Table 1).

To test further the efficiency of the apparatus against soils containing varying percentages of organic matter, 3 replicate pint samples of 1) a muck soil from the bank of a canal, 2) a sandy grass soil, and 3) a sandy soil taken at a 1-ft. depth in a citrus grove were washed for 5, 10, 15, and 20 minutes. The "whole sample" and "tube sample" sieved residues then were immersed in Baermann funnels for 24 hours. The following day, 100 ml was drawn from each funnel, and nematode counts were made on 2 replicate 1-ml aliquots. Average counts thus obtained were amplified to represent the approximate total number of nematodes in each 100-ml sample. Recovery of nematodes varied from 89 to 100 per cent and was as high from organic soils as from soils low in organic matter (Table 2).

The data in Tables 1 and 2 show that a high percentage of recovery of nematodes can be obtained by washing soil samples for only 5 minutes, as determined by comparing counts of nematodes caught on the sieves with counts of the nematodes remaining in the tube. Increasing the washing time to 10 minutes, in most cases, improved the recovery. Further increasing the washing time did not materially increase the efficiency of the washing process.

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LAKE ALFRED, FLORIDA

LITERATURE CITED

1. AHLBERG, O. 1939. Undersökingar över potatisnematoden, Heterodera schachtii Schmidt subsp. rostochiensis Woll. I. Metoder för Kvantitativ bestämning av jordens cystahalt. [Sweden] Statens Växtskyddsanst., Meddel. 29.
2. ANDERSON, E. J., AND I. YANAGIHARA. 1955. A method

for estimating numbers of motile nematodes in large numbers of soil samples. Phytopathology **45**: 238-239.
3. BAERMANN, G. 1917. Eine einfache Methode zur Auffindung von Ankylostomum (Nematoden) Larven in Erdproben. Geneesk. Tijdschr. Nederl. Indië. **57**: 131-137.
4. CAVENESS, F. E., AND H. J. JENSEN. 1955. Modification of the centrifugal-flotation technique for the isolation and concentration of nematodes and their eggs from soil and plant tissue. Helminthol. Soc. Wash. Proc. **22**: 87-89.
5. CHRISTIE, J. R., AND V. G. PERRY. 1951. Removing nematodes from soil. Helminthol. Soc. Wash. Proc. **18**: 106-108.
6. FENWICK, D. W. 1940. Methods for the recovery and counting of cysts of Heterodera schachtii from soil. Jour. Helminthol. **18**: 155-172.
7. OOSTENBRINK, M. 1954. Een doelmatige methode voor het toetsen van aaltjesbestrijdingsmiddelen in grond met Hoplolaimus uniformis als proefdier. [Ghent] Meded. Landbouwhogesch. Opzoekingsst. van Staat Gent **19**: 377-408.
8. OVERGAARD, C. 1948. An apparatus for quantitative extraction of nematodes and rotifers from soil and moss. Natura Jutland. **1**: 273-277.
9. STÖCKLI, A. 1943. Über methoden zur quantitativen Bestimmung der im Boden freilebenden Nematoden. Ber. Schweiz. Bot. Gesell. **53A**: 160-174.
10. STÖCKLI, A. 1950. Über die quantitative Bestimmung der Bodennematoden. Ztschr. Pflanzenernähr, Düngung u. Bodenk. **51**: 1-22.
11. TAYLOR, A. L., AND W. Q. LOEGERING. 1953. Nematodes associated with root lesions in abaca. Turrialba **3**(1-2): 8-13.

FERTILIZING ABILITY OF MALES AND HERMAPHRODITES IN HYPOMYCES SOLANI F. CUCURBITAE [1]

Ralph Baker [2]

SUMMARY

Perithecium primordia in female receptors of *Hypomyces solani* f. *cucurbitae* were spermatized with equal numbers of compatible hermaphroditic and male ascospores. Analysis of individual perithecia 9–17 days after spermatization indicated that at certain times within this period more of the mature perithecia had been fertilized by the male than by the hermaphrodite. This phenomenon is due, at least in part, to the ability of some of the perithecia fertilized by the male to mature at a faster rate than those fertilized by the hermaphrodite.

Several factors influence the relative behavior of the hermaphrodite and male. One of the most important was found to be the fluctuations of light in the laboratory. Consistent results were possible only when environmental conditions were carefully controlled.

Efforts to reproduce the results obtained when mixtures of equal numbers of hermaphroditic and of male ascospores were used for spermatizing, by studying the individual behavior of these 2 strains in fertilizations, were not entirely successful. There is evidence that in cultures grown under suboptimal light conditions, more perithecia are formed when male conidia are used for spermatizing than when hermaphroditic conidia are used. Moreover, perithecia in female receptors growing under the same conditions are brought to maturity at a faster rate.

Four to 8 hours after a 30-day-old female has been spermatized by a hermaphrodite, most of the perithecium primordia were not receptive to the male. Apparently, the step that determines whether a perithecium primordium is to be fertilized by a hermaphrodite or by a male is made rather early in females of this age.

Hypomyces solani f. *cucurbitae* Snyd. & Hans. is a heterothallic ascomycete pathogenic to cucurbits. Although thalli of this fungus are originally hermaphroditic with regard to sex, loss of sex factors is common, with the result that unisexual females and males have been reported (2, 3). The current scope of the research on this fungus has recently been reviewed (7).

The study of sex in *H. solani* f. *cucurbitae* contains several interesting facets, since male and female thalli can be compared genetically and functionally with hermaphroditic thalli. In 1952, Snyder and Hansen reported the preliminary results of such a study on the comparative efficacy of the male and the hermaphrodite in the fertilization of perithecium primordia (6). Their experiments indicated that the male thallus was considerably more efficient in fertilization than was the hermaphrodite. The purpose of this paper is to present the results of further investigations involving this phenomenon.

MATERIALS AND METHODS.—Three sexually different strains of *H. solani* f. *cucurbitae*, representing both mating types or compatibility factors (*A* and *a*), were

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Based on a thesis presented to the Graduate Division of the University of California, Berkeley, in partial fulfillment of the requirements for the degree of Doctor of Philosophy.
Appreciation is expressed to Drs. H. N. Hansen, W. C. Snyder, and Ralph Emerson for their encouragement and assistance.
[2] Assistant Plant Pathologist, Colorado A & M College, Fort Collins, Colorado.

used in this study. Two of these were the unisexual strains (male and female) and the other, the bisexual hermaphrodite. These represent 2 different cultural types. The hermaphrodite and female are of the C (conidial) type and are culturally indistinguishable (3); however, they are readily differentiated from the male or M (mycelial) type by their color and sporodochium formation and by the presence of perithecium primordia (2). In contrast, the M type develops fewer conidia and more abundant mycelium and, because it does not produce perithecium primordia, is incapable of behaving as a female.

Spermatization.—In order to test the comparative action of the hermaphrodite and the male in fertilization, it was necessary that an equal number of the spores of each should be present in the spermatizing material. The use of conidia presented several serious problems among which were the presence of more than 1 spore form and the difficulties involved in attempting to obtain from 2 different thalli an equal number of even-aged fertilizing agents in a spore suspension. This problem was solved through the use of a simple cross, hermaphrodite (A) × male (a), the ascospore progeny of which are known to be in the ratio 1 hermaphrodite : 1 male (2). Twelve days after such a cross was made, the ascospores were used as spermatizing agents. As ascospores began to emerge from the ostioles on the eighth or ninth day, the spermatizing agents were relatively even aged.

Cultures of the female receptor (A compatibility) were started from single spores and grown in 8-in. test tubes on 2 per cent potato-dextrose agar. Culture medium for each experiment was made up at 1 time and adjusted to pH 6.2.

The ascospores to be used for spermatizing were dispersed in sterile water and brought to a concentration of 5×10^6 spores per ml. One-tenth ml of this suspension was sprayed into each culture of the receptor.

Analyses.—Analyses of the progeny resulting from spermatization with equal numbers of hermaphroditic and male ascospores were made on the ninth, eleventh, thirteenth, fifteenth, and seventeenth day after spermatization. Ascospores were produced in some instances on the eighth day, but maturation of perithecia at this time was so sporadic that an adequate sample could not be obtained. No analyses were made after 17 days because of the possible presence of perithecia that had been secondarily fertilized (1).

In the analyses to determine the sex of a fertilizing

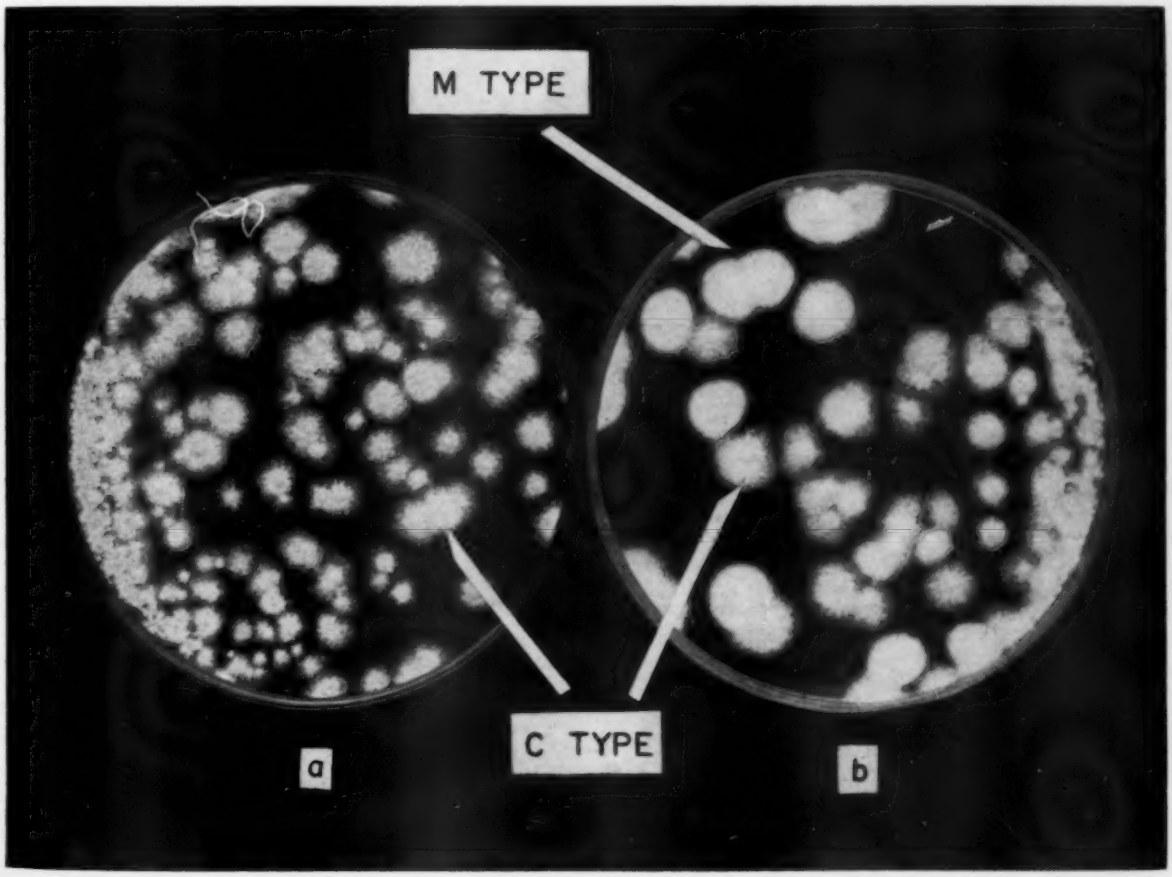

FIG. 1. Progeny resulting from the ascospore progeny of a single perithecium from the cross ♀ × ☿ (a) showing colonies of only the C type and from the cross ♀ × ♂ (b) showing colonies of both C and M types.

agent, the mass of ascospores from the perithecium was removed with a flamed needle under a binocular dissecting microscope and dispersed in sterile water. This suspension was poured into a Petri dish containing acidified potato-dextrose agar. If the fertilizing agent involved were the hermaphrodite, all the colonies developing on the agar were of the C type; however, if the male had acted in fertilization, approximately 50 per cent of the colonies were of the M type (3). The progeny resulting in each of these cases is shown in Figure 1.

In practice, the progenies from 10 or 11 perithecia were analyzed from each of 8–10 tubes, depending upon the size of the experiment. This resulted in a total of 80–110 perithecia being analyzed for any treatment in 1 day.

Sansome (5) has shown that in *Neurospora crassa*, heterokaryon spermatizing agents make it possible for a single perithecium to be fertilized by 2 separate strains. Thus it was thought desirable to test whether multiple fertilization could take place under the conditions of this investigation. Accordingly, mixtures of the conidia of various identifiable strains were used to spermatize female receptors. Twenty-five perithecia were analyzed to determine if more than 1 strain had participated in the fertilization of a single perithecium. In all cases, the characteristics of only 1 spermatizing agent were detectable in the progeny from any single perithecium.

Influence of the age of the receptor.—Groups of 10 cultures (each derived from single spores) of the female were spermatized with equal numbers of hermaphroditic and male ascospores at intervals of 5 or 10 days, beginning at the time the cultures were 15 days old and continuing until 100 days. Each group was analyzed in the prescribed manner to determine the relative efficacy of the hermaphrodite and the male in fertilization at any given age of the female.

The cultures spermatized at 15 and 100 days were divided so that 4 analyses instead of 5 were made in the 2-day intervals from 9–15 days. This was done because very few perithecia were produced in the female cultures of these ages. Perithecium production otherwise was adequate for all samples. Two general tendencies were noted and have been illustrated graphically in Figure 2. The first was demonstrated by the females spermatized when 40 days old or before (Fig. 2, A). Here, only slightly more of the perithecia that matured 9 days after spermatization had been fertilized by the male than by the hermaphrodite. Later, however, the percentage of mature perithecia that had been fertilized by the male strain rose, either eventually reaching a nearly constant level or continuing upward. An exception can be noted in those females that were spermatized when they were 15 days old.

Females 50 days of age or older had the highest percentage of mature perithecia fertilized by the male 9–11 days after spermatization. After that time the percentage decreased, in some cases indicating that eventually the mature perithecia had been fertilized equally by the hermaphrodite and the male. The females spermatized at 100 days are an exception to this general trend.

These results may be analyzed for statistical significance by the method of confidence intervals. Accordingly, if at least 60 per cent of a sample consisted of perithecia that had been fertilized by the male, there is 90 per cent confidence that a greater number of the perithecia in the total population had been fertilized by the male than by the hermaphrodite. This situation occurred at some time in the period of analyses of all spermatizations except those at 15 and 100 days.

Similar tests performed under laboratory conditions are summarized and compared in Figure 3 with

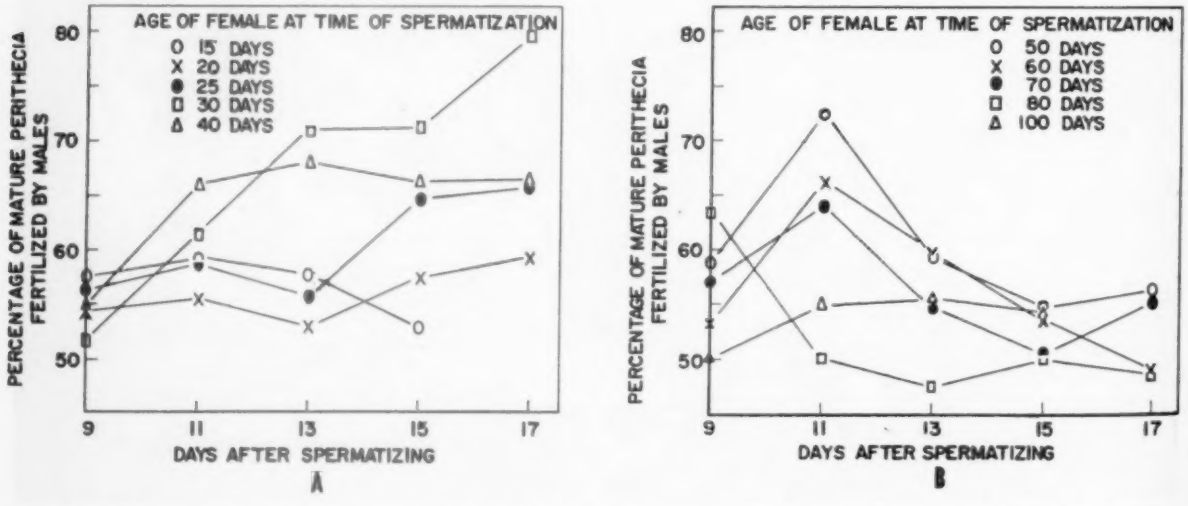

Fig. 2. Percentage of mature perithecia fertilized by males when an equal mixture of hermaphroditic and male ascospores was used to spermatize different-aged females. A) Females 15–40 days old. B) Females 50–100 days old.

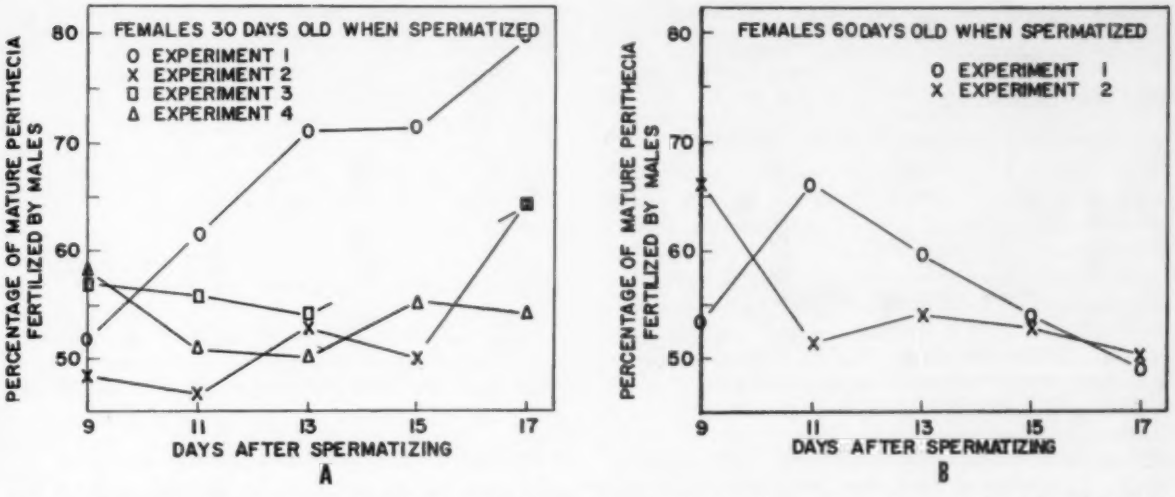

FIG. 3. Summarization of tests conducted when females were spermatized with equal numbers of hermaphroditic and male ascospores at 30 and 60 days under ordinary laboratory conditions. A) Females 30 days old when spermatized. B) Females 60 days old when spermatized.

the results of the experiment described above. Statistical analyses by means of difference in proportions of these repeated trials revealed a significant difference at the 5 per cent level.

INTENSITY OF ILLUMINATION.—The experiments so far described were carried out under ordinary laboratory conditions and were therefore influenced by daily and seasonal variations of light and temperature. Light is not only necessary for primordium formation in *H. solani* f. *cucurbitae*, but it is required for maturation of perithecia after spermatization (3). There is also some evidence that diurnal temperatures above or below the optimum delay the maturation of fruiting bodies. Thus it seemed desirable to test the effect of different intensities of light on the efficiency of fertilization and subsequent perithecium maturation when cultures were spermatized with the different sexual strains.

Thirteen-watt white fluorescent lamps were used to illuminate chambers in which the temperature was held at $25 \pm 1\frac{1}{2}$°C. Illumination was supplied 12 hours each day. The light intensity was measured in foot-candles at the surface of the culture tubes nearest the light source. The intensities used in 3 separate chambers were as follows: 450–460 foot-candles (19 lamps), 190–195 foot-candles (6 lamps), and 36–37 foot-candles (1 lamp).

Female receptors in each of the 3 chambers were spermatized when 25 days old with a suspension of equal numbers of hermaphroditic and male ascospores. The usual analyses were undertaken; samples numbering 80 in each case were taken on the slants between 120–130 mm from the lips of the tubes.

Intensity of illumination had an effect upon the relative behavior of the male and hermaphrodite in fertilization (Fig. 4). A significantly higher percentage of the mature perithecia had been fertilized by males than by hermaphrodites 9 days after spermatization in receptors grown under the lowest light intensity. Again at 11 and 13 days at 190–195 foot-candles, significantly more than 50 per cent of the mature perithecia had been fertilized by the male. Differences at 450–460 foot-candles were of doubtful significance.

An identical test at an illumination of 190–195 foot-candles was performed at a later date. Differences between these results and those of the initial test were not statistically significant at the 5 per cent level.

INDIVIDUAL BEHAVIOR OF HERMAPHRODITE AND MALE THALLI IN FERTILIZATION.—In the chambers described above, parallel spermatizations were made involving

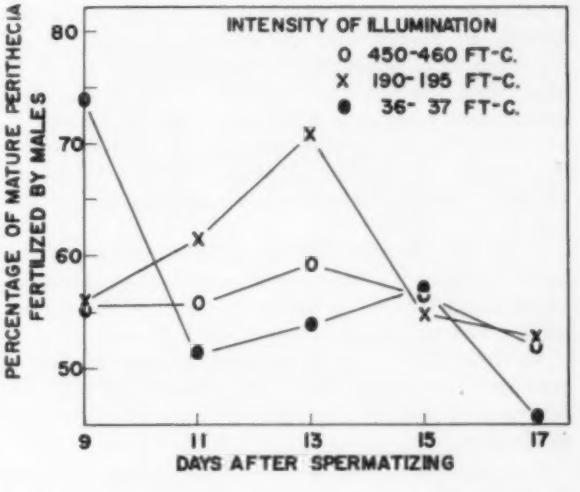

FIG. 4. Percentage of mature perithecia fertilized by males when equal numbers of hermaphroditic and male ascospores were used to spermatize females (25 days old) growing under 3 different light intensities at a constant temperature of $25 \pm 1\frac{1}{2}$°C.

TABLE 1.—*Actual counts*[a] *of the mature perithecia and total number of perithecia in female cultures grown under 3 different light intensities at 25 ± 1½°C and spermatized at 25 days with conidia from either hermaphrodites or males*

Days after spermatizing	450–460 ft.-candles		190–195 ft.-candles		36–37 ft.-candles	
	♀ × ☿	♀ × ♂	♀ × ☿	♀ × ♂	♀ × ☿	♀ × ♂
(Number of perithecia producing ascospores)						
8	16	13	37	54	4	15
9	40	36	56	92	6	40
11	49	50	57	92	15	51
13	58	58	58	100	16	56
15	58	58	62	100	16	57
17	59	64	63	102	18	59
(Total number of perithecia)						
9	212	190	276	264	113	200
17	260	246	314	308	140	260

[a] Counts represent average number in 2 cultures of the receptor of all perithecia included in the area 120–130 mm from the lip of the tube.

the crosses female × hermaphrodite and female × male. Two tubes of each cross were utilized in each chamber, and counts were made from these of the mature perithecia and of the total number of perithecia found in the area included between 120 and 130 mm from the lip of the tube. Perithecia were considered mature when they had produced ascospores. Counts of the mature perithecia were made at 8, 9, 11, 13, 15, and 17 days after conidiation, but counts of the total number of perithecia were made at 9 and 17 days only.

At the 2 lower light intensities, more mature perithecia were produced in the cultures spermatized with male conidia than in those spermatized by hermaphroditic conidia (Table 1). This proved true in similar experiments repeatedly carried out under ordinary laboratory conditions. At the lowest light intensity, more perithecia (mature and immature) were produced in the cultures spermatized by the male than in those spermatized by the hermaphrodite, and the greatest increase of mature perithecia in the cross female × male occurred between 8 and 9 days after spermatization. In comparison, the greatest increase in the cross female × hermaphrodite became apparent at 11 days. This was also characteristic of similar tests performed under ordinary laboratory conditions.

At 190–195 foot-candles, the greatest increase in mature perithecia occurred between 8 and 9 days in both crosses. Further increases were of no great magnitude.

At 450–460 foot-candles, the greatest increase of mature perithecia in both crosses again was noted between 8 and 9 days. A further increase, of lower magnitude, was registered between 9 and 11 days.

As indicated by speed of maturity and number of perithecia produced, the light intensity of 190–195 foot-candles was nearest the optimum for sexual reproduction.

SPERMATIZATION WITH THE MALE AFTER THE HERMAPHRODITE.—An experiment was designed in which females were spermatized first with hermaphrodites and later with males. Thirty-day-old females were spermatized with hermaphroditic conidia only, and then groups of these were sprayed with male conidia at intervals of 2, 4, 8, 16, and 32 hours thereafter.

Because of the excessive number of analyses that would have been required to undertake the usual procedure of sampling perithecia every other day for every treatment, the design of this experiment was somewhat modified. Samples, each of 80 perithecia from 8 cultures, were taken for every treatment at 9, 13, and 17 days after spermatization.

A rather low percentage of fertilization with the male occurred when it succeeded the hermaphrodite in spermatization by 4–8 hours or more (Table 2).

DISCUSSION.—Klebs (4), in summarizing his views on reproduction and growth in fungi, came to the conclusion that optimal environmental requirements exist for each phase of the developmental cycle of a fungus. In *Hypomyces solani* f. *cucurbitae*, variations from the optimum that occur under ordinary laboratory conditions of fluctuating light and temperature apparently have a profound effect on the fertilization process. Evidence of this can be seen in a comparison of the tests carried on under ordinary laboratory conditions and under closely controlled conditions with 25- to 30-day-old females spermatized with equal numbers of hermaphroditic and male ascospores. Reproducible results were obtained in experiments under carefully controlled conditions; in contrast, experiments carried out under fluctuating conditions yielded results which were much more variable.

TABLE 2.—*Percentage of mature perithecia (sample size, 80) that had been fertilized by males when 30-day-old females were spermatized first with hermaphroditic conidia and then at various intervals thereafter by male conidia*

No. of hours between time of spermatization with hermaphrodite and spermatization with male	Percentage of male-fertilized perithecia at indicated intervals following spermatization with male		
	9 Days	13 Days	17 Days
0	57	63	53
4	9	51	33
8	13	10	11
16	8	9	9
32	3	4	9

When a mixture of equal numbers of hermaphroditic and male ascospores was used to fertilize the female receptor used in these tests, it was evident that at certain periods thereafter, more of the resultant perithecia had been fertilized by the male than by the hermaphrodite. Whether this is due to 1) more primordia being fertilized initially by the male than by the hermaphrodite, 2) differential rates in maturity of perithecia fertilized by the different strains, or 3) a combination of these factors is not conclusively proved by these experiments; however, certain conclusions may be drawn.

Evidence for the first hypothesis rests upon the behavior of the receptors when conidiated by either the male or the hermaphrodite. At lower light intensities, more perithecia are produced in receptors fertilized by the male than in those fertilized by the hermaphrodite.

That perithecia are brought to maturity at different rates by males and hermaphrodites when equal numbers of the ascospores of these strains are used for spermatization is quite apparent from a study of the resultant ratios 9–17 days after spermatization. Again in the cross female × hermaphrodite under a low light intensity, perithecia were brought to maturity at a slower rate than in the cross female × male.

The most favorable condition of light intensity for sexual reproduction used in these tests was 190–195 foot-candles. When the female is grown at this intensity of illumination and spermatized with an equal number of hermaphroditic and male ascospores, the perithecia that produce ascospores first are fertilized in equal numbers by each strain; among perithecia producing spores later (13 days after spermatization), those fertilized by the male predominate. In contrast, when old receptors or those growing under a light intensity of 36–37 foot-candles were spermatized in like manner, the first perithecia to produce ascospores (9–11 days) had been fertilized for the most part by the male. Later (13–17 days), the mature perithecia had been fertilized equally by either sexual strain. It is evident that the perithecia fertilized by the male were brought to maturity at a faster rate than many of those fertilized by the hermaphrodite. Thus any difference in the rate of maturation of perithecia by the 2 sexual strains became evident earlier in those cultures in which the perithecium primordia were less vigorous because of senility or suboptimum conditions.

Four to 8 hours after a 30-day-old female growing under laboratory conditions has been spermatized by a hermaphrodite, most of the perithecial primordia apparently have reached a point where they can no longer be fertilized by the male. If this be true, it is likely that the step that determines whether a perithecial primordium is to be fertilized by a hermaphrodite or by a male is made rather early in plasmogamy differences in maturation of perithecia by one strain as compared to another take place later in the sexual process, perhaps in the dikaryotic system. If the genes determining the sexual identity of this fungus do so by controlling the synthesis of specific chemical substances, as proposed by Wheeler (8), it is likely that the relative quantitative amounts of these substances are different in the crosses compared in this investigation.

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LITERATURE CITED

1. El-Ani, A. S. 1952. The genetics of Hypomyces solani f. cucurbitae. Ph.D. Thesis, Univ. of Calif.
2. Hansen, H. N., and W. C. Snyder. 1943. The dual phenomenon and sex in Hypomyces solani f. cucurbitae. Amer. Jour. Bot. **30**: 419-422.
3. Hansen, H. N., and W. C. Snyder. 1946. Inheritance of sex in fungi. Nat'l. Acad. Sci. Proc. **32**: 272-273.
4. Klebs, G. 1900. Zur Physiologie der Fortflanzung einiger Pilze. III. Allegemeine Bet-racht-ungen. Jahrb. f. Wiss. Bot. **35**: 80-203.
5. Sansome, E. R. 1947. The use of heterokaryons to determine the origin of the ascogenous nuclei in Neurospora crassa. Genetica **24**: 59-64.
6. Snyder, W. C., and H. N. Hansen. 1952. Comparative efficacy of male and hermaphrodite in the fertilization of perithecial primordia. (Abs.) Phytopathology **42**: 479-480.
7. Snyder, W. C., and H. N. Hansen. 1954. Species concept, genetics, and pathogenicity in Hypomyces solani. Phytopathology **44**: 338-342.
8. Wheeler, H. E. 1954. Genetics and evolution of heterothallism in Glomerella. Phytopathology **44**: 342-345.

TOBACCO MOSAIC VIRUS IN ORCHIDS IN PUERTO RICO[1]

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SUMMARY

In Puerto Rico, *Cattleya* spp. have been observed with a variety of leaf symptoms suggestive of viral disease. In other instances, Cattleya plants have shown flower breaking without obvious leaf symptoms. When sap from these symptomless leaves was subjected to high-speed centrifugation and the resulting purified preparation injected into rabbits, sera of high titer against the introduced antigen were produced. In precipitin tests, the sera reacted strongly with the preparation and also with purified tobacco mosaic virus.

Various dilutions of the preparation induced typical tobacco mosaic in several solanaceous plants, including 2 varieties of *Nicotiana tabacum*. The virus was not inactivated when heated to 87–90°C for 10 minutes.

Juice from 7 Cattleya plants showing a variety of leaf symptoms was diluted with 0.1 M phosphate buffer and rubbed on tobacco and tomato plants; the resulting symptoms were indicative of tobacco mosaic virus infection. Chloroform-treated extracts of the same crude saps were tested separately with antiserum specific to the preparation mentioned above and with antiserum specific to tobacco mosaic virus. Six samples gave a positive precipitin reaction with the antiserum to the preparation; of these, 4 also reacted positively with tobacco mosaic virus antiserum.

Although the results provide strong evidence of infection of *Cattleya* spp. by tobacco mosaic virus, infection did not appear to be correlated with any particular set of symptoms.

Symptoms suggestive of viral disease have been observed in *Cattleya* spp. in Puerto Rico. In 2 different nurseries, flower breaking without any noticeable symptoms of disease on the leaves of the plant was observed. In another nursery, leaves of individual Cattleya plants were seen to be affected by a variety of symptoms: necrotic spots, chlorotic patches where the leaf was hardened, and light and dark green patches.

Zaitli. et al (6) have shown that symptoms in Cattleya cannot always be relied upon for diagnosis of virus infection. They demonstrated the usefulness of serological tests in detecting virus infection in this species. During the course of precipitin tests carried out with chloroform-treated extracts from orchid leaves, it was found that 1 extract reacted strongly with tobacco mosaic virus antiserum. This study was carried out to determine whether tobacco mosaic virus is present in orchids in Puerto Rico.

MATERIALS AND METHODS.—*Plant material.*—The Cattleya leaf material used for preparation of virus was donated by Mr. G. Jiménez and Mr. F. Méndez of Río Piedras, P. R. This consisted of apparently normal leaves from plants showing flower breaking. The Cattleya leaf material used for tests on individual plants was donated by Dr. Luis J. Fernández of San Juan, P. R.

Extraction and partial purification of virus.—Cattleya leaves of normal appearance were obtained from plants whose flowers had shown the color-breaking symptom. The leaves were ground to a pulp in a food chopper and the sap expressed by subjecting the pulp to a pressure of 1500 lb./sq. in. in a Carver press. The dark viscous liquid obtained was then subjected to 2 cycles of alternate low (4000 g) and high speed (50,000 g) in a Servall SS-2 type centrifuge. High-speed runs lasted for 3 hours. Pellets from the second high-speed cycle were small and darkly colored. They were resuspended in 0.01 M phosphate buffer pH 7.0, equivalent to 1/10 the original volume of expressed sap. The pellets did not dissolve completely, and the undissolved colloidal matter was removed by a third low-speed centrifugation. The final preparation was lightly opalescent and had a faint greenish tinge. The preparation was kept frozen at −20°C and will be referred to as orchid virus preparation (OVP) in this paper. All operations were carried out in a cold room at a temperature of 5°C.

Formalin-treated OVP was shadowed with chromium and examined with the electron microscope.

Determination of nitrogen and of virus concentrations.—Nitrogen determinations were made by the Nesslerization method, and the concentration of virus in OVP and tobacco mosaic virus was calculated by multiplying mg nitrogen by 6.25.[2]

Plant inoculation tests.—Plant species used for inoculation were *Nicotiana tabacum* L. var. Holmes' Samsoun (having the N localization factor from *N. glutinosa* L. (3)), *Nicotiana tabacum* L. var. Virginia-12 (a commercial variety of filler tobacco grown in Puerto Rico, very susceptible to common mosaic), pepper (*Capsicum frutescens* L. var. Large Bell Hot), and tomato (*Lycopersicon esculentum* Mill. var. Marglobe). *Datura metel* L. was used sometimes. Groups of 4 plants of each species were inoculated with 1/5, 1/10, 1/20, and 1/40 dilutions of OVP in 0.01

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We are greatly indebted to Dr. Francis O. Holmes of the Rockebeller Institute for Medical Research for the electron micrographs of the orchid virus preparation.

[2] Thanks are due Dr. C. F. Asenjo, Biochemistry Department, School of Medicine, University of Puerto Rico, for performing the nitrogen determinations.

M phosphate buffer. Leaves were lightly dusted with Carborundum before they were rubbed with a cotton swab dipped in the diluted OVP.

Inoculation of samples from individual Cattleya plants showing different leaf symptoms were made on plants of *N. tabacum* L. var. Holmes' Samsoun, *N. tabacum* L. var. Virginia-12, and *L. esculentum* Mill var. Marglobe. Two plants were used for each sample. Leaf-tip samples (1 or 2 sq. in.) were taken from individual Cattleya plants with leaf symptoms and ground in a clean mortar. The ground material was divided into 2 portions: one was diluted approximately 1/5 with 0.1 M buffer pH 7.0 and used for inoculation; the other was treated by the chloroform method of Zaitlin et al (6). The daytime temperature in the greenhouse during these tests fluctuated between 85° and 92°F.

Preparations of antisera.—OVP was injected into 2 healthy 6-lb. rabbits by both the intraperitoneal and intravenous routes. The doses used were 1 ml intravenous and 2–3 ml intraperitoneally at 4-day intervals. After 4 such injections, the rabbits had developed titers of 1:512 and 1:256, respectively, when tested against OVP. A total of about 7.3 mg OVP protein was injected into each rabbit. Antiserum against tobacco mosaic virus was prepared in a similar manner from a purified preparation of common tobacco mosaic virus isolated locally.

Precipitin tests.—The precipitin method was used for serological testing. Cross testing with OVP and purified tobacco mosaic virus was performed. In tests with leaf-tip extracts of individual Cattleya plants, the chloroform method of Zaitlin et al (7) was used. Each extract was then mixed separately in 4×50-mm tubes with a $\frac{1}{8}$ dilution of each of the following sera: anti-OVP, anti-tobacco-mosaic, and normal rabbit serum. This serum concentration was found most suitable for precipitin testing of the chloroform-treated extracts. After the extracts and antisera were mixed, the tubes were incubated at 37°C for a maximum of 3 hours.

EXPERIMENTAL RESULTS.—*Plant inoculation tests.*—Inoculation of various solanaceous hosts with OVP gave varied results. In the first 2 attempts at virus recovery, very slight symptom response was obtained. Only a few small necrotic spots were observed on inoculated leaves of each of 2 tobacco plants of the Virginia-12 variety inoculated with the 1/5 and 1/10 dilutions of OVP. Also, faint green mottle was observed in the top leaves of these plants. Mosaic mottling was observed also in 2 plants of *Datura metel* L. inoculated with the 1/10 dilution of OVP.

The third and fourth attempts, however, brought a well-defined pattern of symptom development. This was followed for a period of 1 month. The symptoms described below were observed with all tested dilutions of OVP, but they generally were delayed and less intense as dilution increased.

On plants of *N. tabacum* var. Holmes' Samsoun, about 10 to 12 2-mm gray spots more or less circular in appearance were seen on inoculated leaves 2 days after inoculation. At 5 days, the spots had become necrotic, were about 5-mm in size, and showed a light brown margin; the inoculated leaf was slightly deformed or necrosis of the midvein was already apparent. On about the sixth day, appearance of secondary chlorotic spots 1–2 mm in size was observed; there was rapid development of the chlorotic spots into necrotic lesions, and both types were seen in a single leaf. The first necrotic lesions were spreading at this time, and leaf deformation with stem necrosis developed. At 7 days, 1 or more of the uppermost leaves showed numerous necrotic lesions and veinal necrosis. Later, the inoculated leaves were completely necrotized and dead. In about 2 weeks, some plants fell over and died as a result of stem necrosis whereas others recovered. Mottling of the upper leaves was observed sometimes.

Plants of *N. tabacum* var. Virginia-12 developed a slight mottling of the younger leaves about 5 days after inoculation, with vein clearing and chlorosis appearing later. By the tenth day, the definite green mottling and distorting effects of common tobacco mosaic were observed.

About 6 days after inoculation of *L. esculentum* var. Marglobe, a faint chlorotic mottling appeared; vein clearing appeared in about 2 weeks. Some plants showed blistering and distortion, especially of the younger leaves. Definite chlorotic mottling symptoms developed about 1 month after inoculation.

Plants of *C. frutescens* L. var. Large Bell Hot developed some chlorosis and slight wrinkling, especially in the younger leaves, 5 days after inoculation. By 2 weeks, leaf deformation was apparent. Well-defined chlorotic patches and distortion, especially of the younger leaves, were seen about 20 days after inoculation.

Heat-resistance test.—Leaves from 2 plants of *N. tabacum* L. var. Virginia-12 from the above-reported inoculations were ground in a mortar and the crude sap extracted by pressing through gauze. The freshly expressed sap was then placed in 2 small cotton-stoppered test tubes and heated at 87–90°C in a water bath for 10 minutes, after which the tubes were immersed immediately in ice water. The heated sap was then used undiluted for the inoculation of 2 plants each of *N. tabacum* L. var. Holmes' Samsoun, *N. tabacum* L. var. Virginia-12, *C. frutescens* L. var. Large Bell Hot, and *L. esculentum* Mill. var. Marglobe. Replicate inoculations were made. Results were identical to those obtained from the inoculation of the same hosts with unheated OVP.

Precipitin tests with purified preparation.—Relationship between OVP and purified tobacco mosaic virus was shown when each was titrated against a constant concentration of antiserum specific for each virus. The 2 virus preparations reacted identically with antiserum specific for tobacco mosaic virus. Both reacted with anti-OVP serum, although OVP reacted at a 4-fold higher dilution than did tobacco mosaic virus. These results show a close relationship between OVP and tobacco mosaic virus (Table 1).

TABLE 1.—*Reaction in precipitin tests of orchid virus preparation (OVP) and tobacoc mosaic virus (TMV) with antisera specific for each virus*[a]

Serum (1/40 dilution)	Antigen[b]	Reaction[c] at indicated dilution of antigen						
		1/2	1/5	1/10	1/20	1/40	1/80	1/160
Anti-TMV	TMV	++	+	+	+	+	—	—
	OVP	++	+	+	+	+	—	—
Anti-OVP	TMV	++	+	+	—	—	—	—
	OVP	++	+	+	+	+	—	—
Normal	TMV	—	—	—	—	—	—	—
	OVP	—	—	—	—	—	—	—

[a] Antigens and sera diluted in 0.85 per cent saline; 0.2 ml antigen mixed with 0.2 ml serum in 4 × 50-mm tubes; incubation period 2 hours at 40°C, then overnight in icebox.

[b] Antigen concentration was 0.45 mg protein/ml.

[c] A very strong precipitin test is indicated by ++, a fairly strong but weaker positive reaction by +, and no reaction by —.

Tests with individual Cattleya plants.—Plant inoculation tests and precipitin tests with extracts from individual Cattleya plants showing leaf symptoms indicated the presence of tobacco mosaic virus in 4 of 7 such samples (Table 2). The 4 extracts that reacted positively with tobacco mosaic virus antiserum also reacted with OVP antiserum and induced symptoms indicative of infection with tobacco mosaic virus in the test plants. Extracts that reacted with OVP antiserum only also gave rise to similar symptoms; however, complete agreement between the serological and inoculation tests was not found since 1 extract that was negative with both antisera also induced symptoms in the inoculated plants. From these tests, it is clear that the presence of tobacco mosaic virus in Cattleya cannot be associated with any particular symptom in the leaves (Table 2).

Electron microscopy.—Electron micrographs of OVP (Fig. 1) show the presence of both flexuous and straight rods of variable length. The largest fluxuous rod measured about 1080 mμ in length. On the contrary, straight rods as short as 50 mμ also were present.

DISCUSSION.—A review of the literature on the various viral agents described from orchids (1, 2, 4, 5, 6) shows that none of the described agents correspond in morphology to tobacco mosaic virus. Gold (1) described 2 particles, a 480 × 18-mμ sinuous rod and a 300 × 28-mμ sickle-shaped rod, found in electron micrographs of leaves from Cymbidium and Cattleya. Newton and Rosberg (5) reported a 300 × 24-mμ rod and 390 × 21-mμ sinuous particle from orchids showing an abnormal color pattern in the flowers and leaf mottle symptoms. They commented that the shorter rigid form resembled tobacco mosaic virus but was somewhat larger in dimensions. Jensen and Gold (4) described a virus ringspot of Odontoglossum orchid. In electron micrographs of preparations of material from Odontoglossum, they found particles that varied in length from 50 to more than 600 mμ, with a "sharp peak" in the distribution curve at about 280mμ. They stated that of the non-orchidaceous virus particles studied in the electron microscope, those of tobacco mosaic virus appeared to resemble most closely the particles from Odontoglossum.

The above-mentioned morphological descriptions strongly suggest that viruses different from that of tobacco mosaic are involved in the various diseases reported. Thus, prior to the finding in the OVP described herein of rigid rods resembling those of tobacco mosaic virus, there was little evidence on morphological grounds for association of tobacco mosaic

TABLE 2.—*Symptoms on test plants inoculated with extracts from individual Cattleya plants, and the reaction of extracts of the same Cattleya plants in precipitin tests with antisera specific for tobacco mosaic virus and for a purified virus preparation from orchids*

Cattleya leaf No.	Symptoms on leaf	Reaction of chloroform-treated extracts with indicated serum			Symptoms on indicated test plant following inoculation with crude buffer extract		
					Nicotiana tabacum		
		Anti-OVP[a]	Anti-TMV[b]	Normal	Holmes' Samsoun	Virginia 12	Marglobe tomato
1	Hardening with chlorotic patches	+	+	—	LNS; SMM[c]	SCS	CM; D
2	Mosaic and necrosis	+	—	—	LNS; SMM	None	CM
3	Hardening with zones of pale green	—	—	—	TLNS	None	SMM
4	Necrosis	+	—	—	None	None	M; D
5	Necrosis	+	+	—	SMU	SCS	CM
6	Hardening in patches	+	+	—	LNS	None	M; D
7	Hardening with pale green zones	+	+	—	LNS; SMU	SCSN	None
Normal	None	—	—	—	None	None	None

[a] A 1/8 dilution of antiserum specific for orchid virus preparation.

[b] A 1/8 dilution of antiserum specific for tobacco mosaic virus.

[c] Letters indicate the following symptoms: CM = chlorotic mottling; D = leaf deformation; LNS = local necrotic spots; M = mottling; SCSN = small chlorotic spots later becoming necrotic; SCS = small chlorotic spots; SMM = systemic mild mottling; SMU = slight mottling upper leaves; TLNS = tiny local necrotic spots.

virus with orchid viral disease. The findings here reported demonstrate the presence of tobacco mosaic virus in Cattleya orchids. Inoculation of several solanaceous hosts with OVP produced symptoms of tobacco mosaic.

The presence of tobacco mosaic virus in extracts from Cattleya leaves that show a variety of symptoms shows that occurrence of tobacco mosaic virus in

FIG. 1. Electron micrograph of the orchid virus preparation. Approximate magnification 38,800 ×. Note presence of flexuous and straight rods of variable size.

Cattleya cannot be associated, at this time, with definite symptom patterns. Whether tobacco mosaic virus is present in *Cattleya* spp. alone or in combination with 1 or more viruses cannot be discerned from the findings reported.

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LITERATURE CITED

1. Gold, A. H. 1950. Particles, possibly virus, isolated from orchids. (Abs.) Phytopathology **40**: 965.
2. Gold, A. H., and D. D. Jensen. 1952. Some apparent virus relationships in several orchid genera, based on electron microscopy. (Abs.) Phytopathology **42**: 9.
3. Holmes, F. O. 1938. Inheritance of resistance to tobacco-mosaic disease in tobacco. Phytopathology **28**: 553-561.
4. Jensen, D. D., and A. H. Gold. 1951. A virus ringspot of Odontoglossum orchid: Symptoms, transmission, and electron microscopy. Phytopathology **41**: 648-653.
5. Newton, N., and D. W. Rosberg. 1952. Electron-microscope studies of a new orchid virus complex. Phytopathology **42**: 79-82.
6. Zaitlin, M., A. M. Schechtman, J. G. Bald, and S. G. Wildman. 1954. Detection of virus in Cattleya orchids by serological methods. Phytopathology **44**: 314-318.

INCREASING THE SENSITIVITY OF THE LOCAL-LESION METHOD OF VIRUS ASSAY [1]

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SUMMARY

A sensitive local-lesion method of virus assay suitable for use with quantities of purified tobacco mosaic virus as small as 0.01 to 0.005 μg per ml has been developed by use of improved technics and of a combination of factors known to increase the numbers of local lesions on susceptible hosts. These factors are 1) the use of Carborundum uniformly distributed, 2) the use of phosphate buffer as diluent, and 3) the use of *Nicotiana tabacum* var. Xanthi-nc, a new host more susceptible than *N. glutinosa*. The method was found to be approximately 4600 times more sensitive than one involving inoculation, without Carborundum, of *N. glutinosa* with virus suspended in distilled water.

In studies on the intrinsic properties of tobacco mosaic virus (TMV), virus suspensions pure enough to eliminate the protective action of contaminating cell impurities and dilute enough to prevent the mutual protection afforded by concentrated virus must be used. These requirements are met by the assay method reported herein. This method makes it possible to assay dilute virus suspensions containing a fraction of a μg per ml. Each factor affecting the number of local lesions was studied separately, and then the factors found to increase accuracy and sensitivity were combined.

The 2 most important improvements of Holmes's (3) classic local-lesion method of virus assay are undoubtedly the use of comparable opposite half leaves, introduced by Samuel and Bald (4) to increase accuracy, and the use of Carborundum, introduced by Costa (2) to increase sensitivity. Some increase in number of local lesions also has been obtained by suspending the inoculum in phosphate solutions.

Recently a new host, *Nicotiana tabacum* L. var. Xanthi-nc, suitable for local lesion work, has been produced by Drs. R. E. Clausen and D. R. Cameron, Department of Genetics, University of California, Berkeley. The nc or necrotic factor of *N. glutinosa* L. was transferred into *N. tabacum* var. Xanthi, a relatively small-leafed, upright-growing variety. The sensitivity of this variety was compared to that of *N. glutinosa*, and methods of obtaining the largest possible number of local lesions on it were determined.

Materials and methods. — *Strain of virus.* — The original inoculum was an electrophoretically purified strain of TMV with a mobility of 9.0 × 10-5 cm/volt/sec/cm in 0.1 ionic strength cacodylate buffer. Plants of *N. tabacum* were inoculated with it to provide material for subsequent purification. They developed typical tobacco mosaic symptoms.

Purification of virus.—The virus was extracted from 150-g lots of frozen infected tobacco leaves. Partially thawed leaves were passed through a fine-toothed food-grinder, and the juice was pressed through cheesecloth. Enough water was added to the press cake to bring the final volume of the combined extract to 150 ml. The mixture then was pressed again, and the liquid added to the first extract. The virus was purified by 2 cycles of low- and high-speed centrifugation. The first cycle was at 10,000 and 100,000 G and the second at 10,000 and 20,000 G. The last high-

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speed centrifugation was at 20,000 G so that shorter-than-typical TMV particles of low infectivity would not be pelleted. After each high-speed centrifugation, the pellets were taken up in distilled water. The final volume of the virus suspension was made up to 50 ml, and its virus content, according to ultramicro-Kjeldahl analysis, varied from 1.9 to 2.7 mg per ml. Just before inoculation, dilutes to the desired μg/ml were made from this stock. Distilled water was used as diluent unless otherwise indicated.

Local-lesion test plants and their preparation for inoculation.—The test plants, *N. glutinosa* and *N. tabacum* var. Xanthi-nc, were grown in 6-in. pots. Five leaves were left on each plant; the oldest was the third to fifth from the base. All others and the tip were removed. The plants of *N. glutinosa* were prepared in this manner 3 days before inoculation to hasten the expansion of the upper leaves, whereas the Xanthi plants were prepared at the time of inoculation to minimize expansion of leaves. The average area of the Xanthi leaf was 1.7 times that of leaves of *N. glutinosa*. One side of the petiole of each leaf was marked with India ink so that a given inoculum could be applied alternately to the left and right halves. Where possible, inocula were compared on opposite half-leaves, and 4 plants (or 20 half-leaves) were used for each inoculum.

Method of applying Carborundum. — Previous experience showed that Carborundum must be applied evenly over the surface of the leaf in order that subsequent inoculation with virus would produce an even distribution of local lesions. Moreover, where excessive clumping of Carborundum occurred, undue abrasion during inoculation often caused severe injury to the leaf. A dispenser and a method of application were devised that overcame these difficulties. The rim of a 5.5-cm petri dish was rotated on a glass plate covered with a slurry of emery powder. The dish was then filled with 600-mesh Carborundum together with some 2-mm glass beads. A plastic adhesive (Pliobond, Goodyear Tire and Rubber Co.) was applied to the edge of the dish, and a piece of tightly stretched fine-mesh nylon taffeta was sealed on as a cover to serve as a sieve. When the petri dish was inverted about 5 cm from the leaf's surface and gently tapped with a hammer made of a rubber stopper slipped over the end of a plastic rod, the Carborundum was sifted evenly over the surface of the leaf. The dish purposely was held close to the leaf so that Carborundum would not be inhaled by the operator.

Brush method of inoculation.—Paired sets of flat, 2-cm-wide, stiff-bristled paint brushes (Universal 713 No. 10) were trimmed to a bristle length of 1.5 cm and the ends of the bristles evened by touching them lightly to an electric grindstone. Before use, the bristles were softened by soaking in warm water. The inoculum was brushed twice over the surface of the dusted leaves with deliberate, even strokes. When the leaves had dried, the residue was rinsed from the leaves with water. Brushes were washed 3 times with soap and water; this was sufficient to clean them for the next set of inoculations.

TABLE 1.—*Number of local lesions on 20 half-leaves of Nicotiana tabacum var. Xanthi-nc inoculated with tobacco mosaic virus (TMV) with and without Carborundum*

Concentration of TMV in μg/ml distilled water	Lesions on half-leaves inoculated by brushing		Ratio B/A
	A—Without Carborundum	B—With Carborundum	
0.01	4	585	146
0.05	11	1384	126
0.10	36	3582	100

EXPERIMENTAL RESULTS.—*Effect of uniformly distributed Carborundum in increasing the number of local lesions.*—The 20 marked half-leaves of Xanthi were first brushed twice with inoculum. The opposite unmarked halves were then dusted with Carborundum and brushed twice with another portion of the same inoculum. Three concentrations of inoculum, 0.1, 0.05, and 0.01 μg per ml, were used. When no Carborundum was used, there were few local lesions, even with the highest concentration used, 0.1 μg of virus per ml (Table 1). In contrast, numerous lesions resulted, even with the lowest concentration of 0.01 μg per ml, when Carborundum was used. In fact, the greatest gain (146-fold) in local lesions due to use of Carborundum was at this low concentration.

Comparative sensitivity of N. glutinosa and of Xanthi.—Eighty half-leaves of each host were dusted with Carborundum and inoculated with a suspension of 0.05 μg of TMV per ml in distilled water. Five times more lesions per unit leaf area were formed on Xanthi than on *N. glutinosa;* measured by half-leaf ratios, almost 8 times more were formed (Table 2). The upper leaves of *N. glutinosa* tended to form more lesions than the lower ones; the opposite tendency was shown by Xanthi. Aside from its higher susceptibility, Xanthi offers several advantages over *N. glutinosa.* It has larger leaves relatively free of undulations, a sturdy upright habit of plant growth, and satisfactory development under winter conditions of sensitive leaves

TABLE 2.—*Comparative sensitivity of N. glutinosa and N. tabacum var. Xanthi-nc when inoculated with tobacco mosaic virus (0.05 μg per ml of distilled water) by the brush-Carborundum method*

Position of leaf from top to bottom	Number of lesions [a]	
	N. glutinosa	*N. tabacum* var. Xanthi-nc
1	236	1081
2	278	1465
3	263	1410
4	228	2400
5	128	2542
Average per half-leaf	14.8	111.2
Average per 100 sq. cm.[b]	7.8	34.8

[a] Each figure is the total number of local lesions on 16 half-leaves.

[b] Area of leaves on *N. tabacum* var. Xanthi-nc 1.7 times that of leaves on *N. glutinosa.*

suitable for assay purposes.

Method of application of Carborundum.—This method of Carborundum dusting was compared with the method of Beraha et al (1), in which Carborundum is added to the inoculum. Seven per cent by weight of 600-mesh Carborundum was added to inoculum containing 0.01 μg TMV per ml in distilled water, and 20 marked half-leaves of Xanthi were brushed with it. The other halves of the leaves were dusted with Carborundum and brushed with a fresh sample of similar, but Carborundum-free, inoculum. The total number of local lesions formed on the 20 half-leaves inoculated with the inoculum-Carborundum suspension was 336; over 5 times as many, 1701, developed on the dusted half-leaves. Beraha et al used Scotia bean (*Phaseolus vulgaris* L.) for a test plant and a crude virus extract suspended in 0.1 M phosphate buffer of pH 8.5 as inoculum. It appears that the dusting method makes more abrasive particles available at the instant of inoculation than are available when the particles are suspended in the inoculum. This agrees with their observation that the most important factor in increasing infection is the number of abrasive particles present at the time of inoculation.

Phosphate effect.—Both Thornberry (5) and Yarwood (6) found that phosphate caused the greatest increase in infectivity in beans when the pH was 8.5 and the concentration 0.068 M to 0.1 M (K_2HPO_4); however, with dilute suspensions of purified TMV, such a high pH causes appreciable inactivation of the virus within a short time. The highest pH at which the phosphate effect safely could be measured was pH 7.0. In these experiments, a suspension containing 20 μg of TMV per ml was diluted with an equal volume of 0.1 M phosphate buffer at pH 7.0. This suspension, along with a control series diluted with distilled water, was stored at 5°C for 4 hours, then diluted with the appropriate diluent to 0.01 μg TMV per ml.

On the 20 Xanthi half-leaves inoculated with the virus in distilled water, the total number of local lesions formed was 651; on the opposite 20 half-leaves inoculated with virus in phosphate, there were 1314 lesions. The 2-fold increase due to phosphate is small compared with the 100-fold obtained by Yarwood with bean leaves. Xanthi, like *N. glutinosa*, apparently is much less susceptible to the action of phosphate than is bean.

Combined effect.—In a final experiment, the combined effect of the 3 factors that were found to increase the number of local lesions, namely, 1) use of evenly applied Carborundum, 2) a more susceptible host, and 3) use of phosphate buffer, was tested. Xanthi was used as the more susceptible host; *N. glutinosa* was included for comparison. One half of each leaf was brushed with a suspension containing 0.01 mg of TMV per ml. The other halves were dusted with Carborundum and brushed with a 0.05 M phosphate buffer (pH 7.0) containing 0.01 μg of TMV per ml, an amount calculated to produce approximately the same number of lesions as anticipated on the other halves of the leaves.

TABLE 3.—*Increased lesion formation resulting from use of a sensitive test plant, use of Carborundum, and use of phosphate buffer as a diluent*

Exp.	Test plant	Number of lesions on half-leaves inoculated by brushing by indicated method with indicated concentration of TMV		Relative sensitivity of Method B in terms of that of Method A[c]
		Method A[a] (0.01 mg/ml)	Method B[b] (0.01 μg/ml)	
1	*N. glutinosa*	495	674	1360
	N. tabacum var. Xanthi-nc	1629	2048	1260
2	*N. glutinosa*	442	619	1400
	N. tabacum var. Xanthi-nc	1863	2249	1210
Relative sensitivity of Method B on *N. tabacum* var. Xanthi-nc in terms of those of Method A on *N. glutinosa*				4583

[a] Distilled water was used as diluent; half-leaves inoculated without Carborundum.

[b] 0.05 M phosphate buffer (pH7) used as diluent; half-leaves dusted with Carborundum.

[c] 1000 × number of lesions by Method B divided by number of lesions by Method A.

[d] 1000 × number of lesions by Method B on *N. tabacum* var. Xanthi-nc divided by number of lesions by Method A on *N. glutinosa*.

Through the combined use of Carborundum and phosphate, the sensitivity of both *N. glutinosa* and Xanthi was increased 1200–1400 times (Table 3). The improved method used with Xanthi increased the sensitivity over the old method on *N. glutinosa* approximately 4600-fold.

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LITERATURE CITED

1. BERAHA, L., M. VARZANDEH, AND H. H. THORNBERRY. 1955. Mechanism of action of abrasives on infection to tobacco mosaic virus. Virology **1**: 141-151.
2. COSTA, A. S. 1944. Quantitative studies with carborundum and its use in local lesion tests. Phytopathology **34**: 288-300.
3. HOLMES, F. O. 1929. Local lesions in tobacco mosaic. Bot. Gaz. **87**: 39-55.
4. SAMUEL, G., AND J. G. BALD. 1933. On the use of primary lesions in quantitative work with two plant viruses. Ann. Appl. Biol. **20**: 70-90.
5. THORNBERRY, H. H. 1935. Effect of phosphate buffers on infectivity of tobacco mosaic virus. Phytopathology **25**: 618-627.
6. YARWOOD, C. E. 1952. The phosphate effect in plant virus inoculations. Phytopathology **42**: 137-143.

KINETICS OF THERMAL DESTRUCTION OF CITRUS TISSUES IN RELATION TO THE VIRUS DISEASE PROBLEM[1]

W. C. Price and L. C. Knorr

SUMMARY

Thermal destruction of citrus buds was studied by immersing short pieces of stem in a water bath and observing necrosis on or near the bud. Psorosis and tristeza viruses both survived heat treatment as long as the buds survived over the range 35–52°C; psorosis virus survived 45°C for 240 minutes and 52°C for 10 minutes; tristeza virus survived 35°C for 3 days, 45°C for 180 minutes, and 52°C for 30 minutes. Thermal killing of citrus buds apparently results from thermal destruction of a protein or enzyme system within cells of the bud. The heat of activation of the sensitive molecules was found to be 66,854 calories per mole, a tentative value based on a specific assumption. Rates of survival of the buds indicated that more than one sensitive molecule per cell, and/or more than one cell per bud, must be destroyed to kill the bud. It was calculated that this number might be as large as 1000.

Knowledge of the physical properties of viruses that cause disease in citrus is lacking, mainly because they cannot currently be transmitted by mechanical means. Conceivably, one or another of them could be more thermolabile than their host tissues, as are a few other plant viruses[2,3,4]. To investigate this possibility, we studied the resistance of citrus tissues to heat and used the information thus acquired in attempts to destroy the viruses of tristeza and psorosis.

It would be possible to learn something of the thermostability of citrus tissues from experiments carried out primarily to discover whether virus can survive in tissues heated at various temperatures for periods less than those required to destroy the host, but only large numbers of such experiments would yield data useful in determining death rates. Experiments were therefore designed to determine rates of survival of citrus tissues over a 17°C temperature range. In this paper, we shall develop theoretical concepts for survival of multicellular tissues exposed to high temperatures, present the actual results obtained with such tissues, and describe experiments in which tristeza and psorosis viruses survived in host tissues exposed to high temperatures.

MATERIALS AND METHODS.—Four species of citrus were chosen for experimentation: Tahiti lime (*Citrus aurantifolia* (Christm.) Swing.), rough lemon (*C. limon* (Linn.) Burm. f.), sweet orange (*C. sinensis* (Linn.) Osbeck), and grapefruit (*C. paradisi* Macf.). Wood was selected, when required, from vigorous mature grove trees. In one experiment, we found that the young willowy growth at the tip of branches was more sensitive to heat than older wood 0.8 cm or less in diameter; in all subsequent experiments, this willowy growth was discarded and only the older stems used. Some of the factors over which we exercised no control in selecting budwood were variations due to season, fertilizer program, and individual trees; one or more of these variations possibly may be reflected in the variations found in the results.

After all leaves were removed from the budwood, it was cut into pieces about 3 in. long. These pieces were assorted randomly into bundles of about 30 sticks and immersed in a hot water bath. The bath was thermostatically controlled and of sufficient volume that its temperature was lowered by less than ½°C when bundles of budwood were immersed in it. One bundle was kept in water at room temperature while the others were being heated; it served as the control. The heating period was terminated abruptly by immersing budsticks in fresh tap water immediately after removal from the bath.

Several methods were tried for preserving budsticks until effects of the hot water treatment could become apparent. The one finally adopted was to immerse the sticks in a 1.0 per cent solution of 8-hydroxyquinoline sulfate for a few minutes to prevent growth of fungi and then to roll them into a ragdoll made of cheesecloth that had previously been treated with 8-hydroxyquinoline sulfate. The ragdolls were kept moist either by holding them in a moist chamber or by wetting them by means of an atomizer every few days. It was found necessary to unroll the ragdolls at intervals of 2–3 days and expose the sticks to air to prevent death due to asphyxiation.

During the first few experiments, it became apparent that selection of criteria for judging whether treated tissues died or remained alive constituted a problem. Survival of budwood when grafted into suitable seedlings would have served as a good criterion but was not attempted because of the time and material that would have been necessary. Sprouting of buds from sticks stuck in moist sand or in vermiculite was tried but abandoned because of erratic sprouting, even

[1] Accepted for publication July 13, 1956.
Florida Agricultural Experiment Stations Journal Series, No. 490.

[2] Kunkel, L. O. 1936. Heat treatments for the cure of yellows and other virus diseases of peach. Phytopathology 26: 809-830.

[3] Martin, J. P. 1933. Pathology. (A report). Hawaiian Sugar Planters' Assoc. Exp. Sta. Rpt. (1932). p. 24-35.

[4] Wilbrink, G. 1923. Warmwaterbehandeling van stekken als geneesmiddel tegen de sereehziekte van het suikerriet. Arch. v. Suikerindus Nederland. Indië Meded. Proefsta. v. Java-Suikerindus 31: 1-15.

among the controls, and because of rotting of the buried portion of the stems. Another characteristic tried and abandoned as a sole criterion, but still found useful as a supplementary aid in diagnosis, was failure of killed budsticks to drop petioles; petioles on control sticks usually dropped after 4 or 5 days in the ragdoll, but this varied with the kind of citrus and was occasionally erratic. The criterion of death finally adopted was the development of small patches of necrotic cells in the immediate vicinity of a bud or on the bud itself. Necrotic areas, including those visible with a 12× dissecting microscope, were recorded. These patches became visible in a few days as yellow or tan areas on Tahiti lime and rough lemon; in severe cases, they covered the entire stem. The lesions had a water-soaked, olivaceous appearance on grapefruit and sweet orange. Final readings were taken when budsticks had been in the ragdoll for 8–10 days.

THEORETICAL CONSIDERATIONS.—The thermal destruction of bacteria has been discussed in detail by Rahn[5], who postulated that destruction of a single gene is sufficient to cause death of a single bacterium. The theory is given a much more elegant treatment by Johnson, Eyring, and Polissar[6], who considered also the case for multicellular organisms. The type of survival curve to be expected when citrus buds, or other multicellular organs, are exposed to heat can be deduced from the hypothesis advanced by these authors.

Let it be assumed that death of a particular cell results from destruction of a definite number of molecules and that destruction of the molecules obeys the mass law. The probability of survival, q, of a particular molecule is the same as the proportion of molecules that survive and is given by

$$q = m_t/m = e^{-k't}$$

where m is the total number of sensitive molecules in the cell, m_t the number surviving at time t, and k′ the reaction velocity constant. The proportion of molecules destroyed will be

$$p = 1-q = 1 - e^{-k't}.$$

If there is only one sensitive molecule in the cell and if the destruction of only this sensitive molecule will lead to death of the cell, then Q, the probability of survival of the cell, is the same as q, the probability of survival of the molecule. If, however, the number of sensitive molecules (m) is greater than 1 and if n of the m molecules must be destroyed to kill the cell, then Q is given by the last n terms in the expansion of the binomial

$$1 = (p + q)^m.$$

This is true because the sum of the last n terms of the binomial expansion represents the proportion of cells in which the number of sensitive molecules destroyed is less than n whereas the sum of the remaining terms represents the proportion in which the number destroyed is n or greater.

When n = 1,

$$Q = q^m = e^{-mk't}.$$

Thus, the slope of the line relating logarithms of the surviving cells to time is proportional to m. This line departs from linearity as n increases in value.

As an illustration, consider the simple case where m = 3 and n = 2:

$$1 = (p + q)^3 = p^3 + 3p^2q + 3pq^2 + q^3.$$

In this case, p^3 is the proportion of cells in which all 3 sensitive molecules are destroyed, $3p^2q$ the proportion in which 2 of the 3 molecules are destroyed, $3pq^2$ the proportion in which 1 molecule is destroyed and 2 survive, and q^3 the proportion in which all 3 molecules survive. Consequently,

$$Q = 3pq^2 + q^3 \text{ and}$$
$$P = 1 - Q = 1 - 3pq^2 - q^3,$$

where P is the probability that the cell will be killed. Substituting $e^{-k't}$ for q and $1 - e^{-k't}$ for p,

$$Q = 3e^{-2k't} - 2e^{-3k't}$$

and

$$P = 1 - 3e^{-2k't} + 2e^{-3k't}.$$

Now, consider a multicellular organ, such as a bud, containing *a* cells of which *b* must be killed to destroy the organ. The probability, S, that the organ will survive is given by the last *b* terms in the expansion of the binomial

$$1 = (P + Q)^a.$$

To continue with the simple example, in which m = 3 and n = 2, let *a* = 3 and *b* = 2. Then

$$1 = (P + Q)^3 = P^3 + 3P^2Q + 3PQ^2 + Q^3$$

and

$$S = 3PQ^2 + Q^3.$$

Substituting the above-mentioned values of P and Q in this equation, we find that

$$S = 27e^{-4k't} - 36e^{-5k't} - 42e^{-6k't} + 108e^{-7k't} - 72e^{-8k't} + 16e^{-9k't}.$$

It is obvious that computation of survival values for even such a simple case as this is laborious; as m and n or *a* and *b* increase in size, such computation becomes impractical. Of course, if death of individual cells depends upon destruction of only a single sensitive molecule, as demonstrated for certain kinds of bacteria[5], then Q = q and S is easier to calculate; e.g., when n = 1, *a* = 3, and *b* = 2, $Q = q = e^{-mk't}$ and $S = 3e^{-2mk't} - 2e^{-3mk't}$. But *a*, the total number of sensitive cells in a bud, and *b*, the number that must be destroyed to kill the bud, are likely to be large so that computation of S would still be laborious. In addition, it is not unlikely that different cells in the organ—because of difference in position, in pH of their sap, or in other respects—will have different reaction velocity constants. The proportion of survivors could then be calculated only by summing the curves obtained for these different velocity constants. For all these reasons, it appears impractical to attempt an evaluation of k′ by fitting experimental survival curves strictly in accordance with the theoretical considerations outlined above. The question we shall attempt to answer is this: can a reasonable estimate of k′ be

[5] Rahn, O. 1945. Injury and death of bacteria by chemical agents. Biodynamica, Normandy, Missouri. 183 p.

[6] Johnson, F. H., H. Eyring, and M. J. Polissar. 1954. The kinetic basis of molecular biology. Wiley, New York. 874 p.

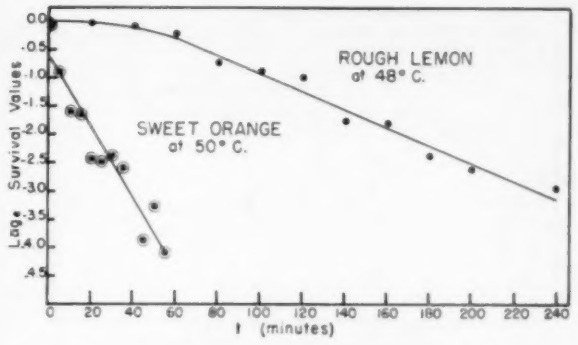

FIG. 1. Selected data illustrating relationship of bud survival to time of heating in a water bath. The upper curve is representative of the type obtained in about ⅔ of the tests; the lower curve is representative of the type obtained in the remainder.

obtained by a method of approximation?

RATES OF SURVIVAL OF CITRUS BUDS.—We carried out 39 experiments in which stems of 4 species of citrus were immersed in water at temperatures of 35–57°C. When the logarithms of the numbers of surviving buds were plotted against time in minutes, curves such as those in Figure 1 were obtained. The upper curve in this figure resembles that to be expected when death of the bud depends upon death of more than a single cell within the bud. There was a lag period during which a relatively small proportion of buds was killed, followed by a period during which survival of buds appeared to be exponential. In the lower curve of Figure 1, there was no detectable lag period; the survival rates decreased exponentially during the entire period. This is the sort of curve to be expected when $n = 1$ and $b = 1$, but this cannot be interpreted to mean that destruction of a single sensitive molecule is all that is necessary to kill the bud. The number of buds that were treated is infinitely small in comparison with the number of molecules usually employed in studying, for example, thermal denaturation of proteins; this small number of buds, coupled with an apparently high degree of variability among buds, probably accounts for failure to detect a definite lag period in this test.

Results with the remaining 37 tests were similar; in about ⅔ of the tests there was a detectable lag period. In each test, the straight-line portion was fitted to the corresponding points, by the method of least squares, according to the equation:

$$\ln S_t = \ln S + Kt$$

where S_t is the survival rate at time t minutes, ln S is the intercept at time 0, and K is the slope.

If K (the reaction velocity constant for killing of citrus buds) is identical with k′ (the reaction velocity constant for destruction of sensitive molecules in the bud cells), then K might be expected to vary with temperature according to the Arrhenius equation

$$K = Ae^{-E/RT}.$$

This equation may be written

$$\ln K - \ln A = -(E/R)(1/T).$$

Thus, K should be a linear function of the absolute temperature of the water bath, and a graph of this function will have the slope $-E/R$ and the intercept ln A.

When the logarithms of the K values obtained for Tahiti lime, rough lemon, sweet orange, and grapefruit were plotted as ordinates and 1/T as abscissas, the points fell along straight lines that did not differ significantly in slope $(-E/R)$ but that did differ in intercept (ln A). Consequently, the data were replotted as in Figure 2, using a combined slope obtained by the method of least squares. Despite the fluctuation of the points about the line, analysis of variance revealed no significant indication of a curvilinear relation between ln K and 1/T. Therefore, the data are consistent with the hypothesis that K is identical with k′.

We know, however, that the same relationship would be obtained if K is a simple multiple of k′. Let m be a constant so that $mk' = K$. Then $\ln K = \ln m + \ln k'$. If this equality now be substituted into the Arrhenius equation, the slope will be the same but the intercept becomes $(\ln A - \ln m)$ rather than ln A. Thus, the energy of activation, which is calculated from the value obtained for slope, is the same whether $K = k'$ or $K = mk'$. Since a value of 33,655 was obtained for the combined slope, the energy of activation was calculated to be 66,854 calories per degree per mole.

It may be instructive to consider the data from the viewpoint of the theory of absolute reaction rates[6]. The molecular destruction responsible for death of the citrus-bud cells results from a rate process in which there is a quasi-equilibrium between an active state and the native state. The quasi-equilibrium constant, designated by $K^{\ddagger}$, has the value

$$K^{\ddagger} = k'/(\kappa\, kT/h),$$

in which κ is the transmission coefficient (usually taken as unity), k is the Boltzmann constant, and h is Planck's constant. As pointed out by Johnson, Eyring, and Polissar[6],

$$K^{\ddagger} = e^{-\Delta F^{\ddagger}/RT} = e^{-\Delta H^{\ddagger}/RT}\, e^{\Delta S^{\ddagger}/R},$$

in which $\Delta F^{\ddagger}$, $\Delta H^{\ddagger}$, and $\Delta S^{\ddagger}$ are the standard free energy, heat, and entropy of activation, respectively.

The slope of the Arrhenius equation, E/R, is approximately equal to $\Delta H^{\ddagger}$. Since it depends entirely upon the change in K with temperature, 66,854 calories per degree per mole is presumed to be the heat of activation of the sensitive molecules within individual cells, if one assumes either that there is only one species of molecule involved in thermal destruction of citrus cells or that the experimentally determined rate constants represent means of a variety of k′ values. This seems to be a reasonable assumption in view of the fact that all 4 species of citrus yielded, within the limits of experimental error, the same energy of activation.

The computed values of A for the 4 sets of data, corrected for the fact that the K values of Figure 2 are in terms of reciprocal minutes rather than reciprocal seconds, are 96.637, 96.162, 95.737, and 95.077 for

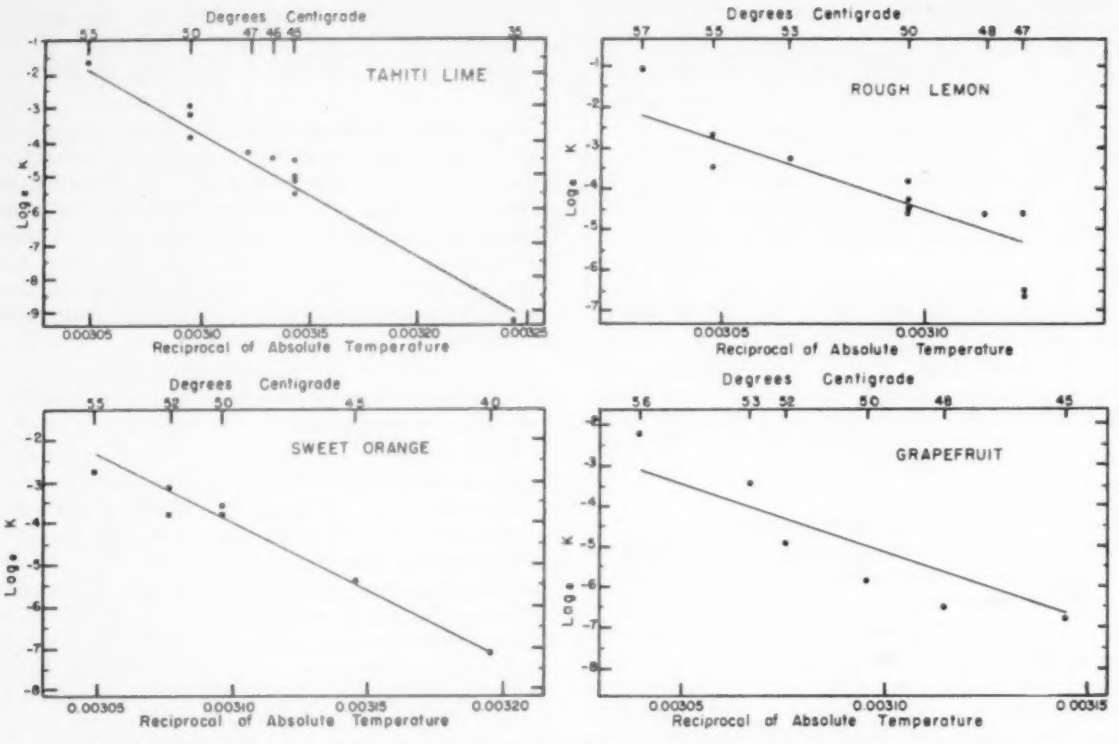

FIG. 2. Plot of logarithms of specific reaction rate constants for thermal killing of citrus buds (K) as a function of the reciprocal of the absolute temperature of the water bath. The theoretical lines drawn through the 4 sets of points have identical slope, all 39 points being used to compute the slope.

Tahiti lime, sweet orange, rough lemon, and grapefruit, respectively; these values reflect a decrease in sensitivity of the citrus species, in that order, to hot water treatment. Expressed in another way, Tahiti lime buds would be expected to die as rapidly at 41°C, on the average, as grapefruit buds at 45°C.

Results with Tahiti lime buds were such as to indicate a more rapid rate of killing of buds on stems taken during fall and winter than during spring and summer. Analysis of variance of the data for all 4 species of citrus failed, however, to bring out such a differential. The observed variation in rate from test to test cannot, therefore, be attributed to a seasonal effect.

The A of the Arrhenius equation is approximately equal to $(\kappa\, kT/h)\, e^{\Delta S^{\ddagger}/R}$. From this approximate equivalence, $\Delta S^{\ddagger} = R(\ln A - \ln(\kappa\, kT/h))$. If we assume momentarily that $k' = K$, it is possible to calculate $\Delta S^{\ddagger}$ from the present data and then to calculate $\Delta F^{\ddagger}$ from the relationship

$$\Delta F^{\ddagger} = \Delta H^{\ddagger} - T\Delta S^{\ddagger}$$

The calculated values of $\Delta H^{\ddagger}$, $\Delta F^{\ddagger}$, and $\Delta S^{\ddagger}$ at 50°C are in Table 1. The values for $\Delta F^{\ddagger}$ are of the same order of magnitude as those observed generally for proteins and enzymes.[6]

If, as suggested earlier, $K = mk'$, the calculated values of ln A given above would need to be diminished by ln m. When m is taken to be 1000 and the calculated values of ln A are diminished by ln 1000, or 6.090, values for $\Delta F^{\ddagger}$ of about 28,400 are obtained. This value is still of the same order of magnitude as those determined for proteins and enzymes. Our results are not, therefore, inconsistent with the hypothesis that as many as 1000 cells must be killed in order to kill the bud, and/or that more than one sensitive molecule must be destroyed to cause death of the cell.

FAILURE TO INACTIVATE TRISTEZA AND PSOROSIS VIRUSES IN VIVO.—In the planning of experiments on the ability of tristeza and psorosis viruses to survive heating *in vivo*, consideration was given to the possibility that a plot of the reaction velocity constant for thermal inactivation of the virus as a function of 1/T would give a line that would cross that for the citrus

TABLE 1.—*Calculated values for heats of activation, free energies of activation, and entropies of activation involved in thermal destruction of citrus budwood*[a]

Species	$\Delta H^{\ddagger}$ (Cal.)	$\Delta F^{\ddagger}$ (Cal.)	$\Delta S^{\ddagger}$
Tahiti lime	66,854	23,782	133.31
Sweet orange	66,854	24,085	132.37
Rough lemon	66,854	24,357	131.53
Grapefruit	66,854	24,780	130.22

[a] The values for $\Delta F^{\ddagger}$ and $\Delta S^{\ddagger}$ are quite arbitrary because they are based on the assumption that $K = k'$ whereas the data actually suggest that K is a simple multiple of k'. The value for $\Delta H^{\ddagger}$ is less arbitrary.

TABLE 2.—*Survival of Marsh Seedless grapefruit budsticks from a psorosis-affected tree after being heated in a water bath then grafted into Pineapple sweet orange test seedlings, and transmission of psorosis virus from the treated budsticks to the test seedlings*

Temperature	Time (minutes)	No. of budsticks surviving of 12 grafted	No. of test plants developing psorosis
45°C	0	4	9
	30	6	10
	60	9	8
	120	3	6
	180	5	7
	240	0	1
52°C	0	8	3
	10	2	1
	20	0	0
	30	0	0

TABLE 3.—*Survival of Valencia sweet orange budsticks from a tristeza-affected tree after being heated in a water bath then grafted into key lime seedlings, and transmission of tristeza virus from the treated budsticks to the test seedlings*

Temperature	Time	No. budsticks surviving of no. grafted	No. of test plants developing tristeza
	Days		
35°C	0	9/20	6
	1	4/10	3
	2	3/10	1
	3	1/10	1
	4	0/10	0
	Minutes		
45°C	0	6/12	7
	30	8/12	8
	60	6/12	6
	120	7/12	5
	180	2/12	2
	Minutes		
52°C	0	6/12	9
	10	7/12	7
	20	5/12	6
	30	1/12	2

host or would lie above it over the range 35–55°C. This would mean that at one end or the other of the temperature range, the rate of inactivation of the virus would be greater than the rate of killing of the host buds. If this were not so then the virus could not be expected to inactivate completely *in vivo*.

Budsticks from a Marsh seedless grapefruit tree infected with psorosis virus were heated in a water bath at 45° and at 52°C, and budsticks from a Valencia orange tree infected with tristeza virus were heated at 35°, at 45° and at 52°; both trees were growing on rough lemon rootstock. After various periods of heating, the budsticks were removed, cooled, and side-grafted into Pineapple sweet orange seedlings to test for survival of psorosis virus and into Key lime seedlings to test for survival of tristeza virus. Test seedlings were observed during 6 months for survival of the grafts and for transmission of virus. From the data thus obtained (Tables 2, 3), it is concluded that both viruses survived as much treatment with heat as would permit the budsticks to survive. Indeed, as can be seen from the data, both viruses were transmitted occasionally to test seedlings by budsticks that failed to survive in the test plants. In such cases, it is presumed that the budstick remained alive sufficiently long to permit transmission in the absence of a firm union. This raises the question of whether an actual union between stock and scion is essential for transmission of psorosis and tristeza viruses or whether prolonged contact between diseased and healthy tissues is sufficient. The data do not provide an answer to this question. The rate of killing of citrus tissues as measured by failure to form a graft union was somewhat greater than the rate measured by necrosis of the bud cells. No particular significance is attached to this differential at the moment because of the small number of tests involved. Yet it is possible that the tissues essential to formation of a union are more sensitive to heat than are those of the bud and surrounding tissues.

It may be of value to speculate briefly about the significance of failure to inactivate either tristeza or psorosis virus *in vivo* by hot water treatment. Survival of the viruses under the time-temperature conditions *in vivo* of 10 minutes at 52°C, 180 minutes at 45°, and 3 days at 35° suggests that they might also survive *in vitro* under similar conditions. Thus, neither virus may be so instable *in vitro* as to preclude possibility of transmitting it mechanically from host to host or of concentrating it by high-speed centrifugation or other physical or chemical means.

CITRUS EXPERIMENT STATION
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THE EFFECT OF THE PERCENTAGE OF SEED TRANSMISSION UPON SUBSEQUENT SPREAD OF LETTUCE MOSAIC VIRUS [1]

F. W. Zink, R. G. Grogan, and J. E. Welch [2]

SUMMARY

The percentage of lettuce plants that develop common lettuce mosaic in the field is largely dependent upon the amount of seed-borne virus and upon the number and motility of the aphid vectors. The degree of disease control resulting from the use of seed that gave small percentages of seed transmission was variable, but in practically every instance, as the amount of infected seed was reduced the total percentage of plants that became infected also was reduced. The data from all trials indicated that if the percentage of seed transmission exceeds 0.1 per cent, the degree of control is likely to be unsatisfactory.

The efficacy of virus-free lettuce seed for control of common lettuce mosaic was reported by Grogan et al[3] in 1952 and has been demonstrated in other tests in subsequent years. These demonstrations have created a strong demand for virus-free lettuce seed, and commercial seed companies have attempted to satisfy this demand. Unfortunately, some of the attempts to produce virus-free seed on a commercial scale have not been completely successful. As a consequence, only limited supplies of virus-free, or nearly virus-free, seed have been available; however, considerable lettuce seed has been sold that was labeled to indicate that the seed-borne virus content was lower than that usually present in ordinary commercial seed stocks. For example, some commercial seed stocks have been labeled as containing less than 0.1 per cent seed-borne virus, others less than 0.33 per cent, whereas still others have been labeled simply "low mosaic count." The disease control that has resulted from the use of some of these commercial seed lots has been unsatisfactory. This indicated a need for more exact information concerning the effect of different percentages of seed transmission upon subsequent spread of the virus and upon the total percentage of infected plants.

MATERIALS AND METHODS.—The highest percentage of seed transmission studied was from a commercial seed stock of the variety Great Lakes 6238; in a greenhouse index, 1.6 per cent of 8133 seedlings were infected. To obtain seed stocks with percentages of virus-seed transmission intermediate between 1.6 per cent and the 0.0 per cent transmission of virus-free seed, the commercial seed stock was diluted with virus-free seed of the same variety. The dilutions were increased stepwise by a factor of 2 so as to prepare seed stocks that were expected to transmit the virus in approximately 0.8, 0.4, 0.2, 0.1, 0.05, and 0.025 per cent of the cases. The highly diluted samples with the smallest expected percentages of seed transmission were not indexed because of the extremely large number of seedlings that would have been required to obtain a reliable index figure. Indexes of approximately 3000 seedlings of each of the 2 dilutions that were expected to contain 0.8 and 0.4 per cent infected seed, however, were found to agree closely with the expected percentages. It was assumed, therefore, that the expected percentages of seed transmission of the higher dilutions were also correct, or at least approximately so.

Eight plantings of the above-mentioned seed stocks were made in commercial lettuce fields in the Salinas Valley in 1955. Each of the 8 seed stocks was planted in a space 12 beds (40 ft.) wide by 50 ft. long that produced from 807 to 998 plants in each of the plots after thinning. The arrangement of plots in each trial was end on end, but the ends and beginnings of adjacent treatments were separated by blocks (20-ft. long and 12-beds wide) planted with virus-free seed. A border 4-beds (13.3 ft.) wide along the entire length of both sides of each trial was planted with virus-free seed. The plots within any 1 trial were not replicated. To facilitate comparisons between low and high levels of seed transmission of virus and also to serve as a check on aphid distribution and activity, all trials contained the following pairing of plots: 1.6 per cent infected seed with virus-free seed, 0.8 with 0.025, 0.4 with 0.05, and 0.2 with 0.1. Each planting was placed at least 200 ft. from the edge of the commercial field. Care was taken to select locations that did not have old lettuce fields immediately upwind and to select fields with beds that ran parallel to the prevailing wind.

The mosaic-affected plants in each plot were staked and mapped at 3 different times during the growth of the crop. The first recording was made 7–14 days after thinning, the second 14–22 days after the first, and the final reading just prior to the time of first harvest.

Effect of the percentage of infected seed upon subsequent spread of the virus during growth of the crop.—The total percentage of plants that became infected in each of the 8 trials was dependent to a large extent

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[3] Grogan, R. G., J. E. Welch, and R. Bardin. 1952. Common lettuce mosaic and its control by the use of mosaic-free seed. Phytopathology 42: 573-578.

TABLE 1.—*Total percentages of lettuce plants with lettuce mosaic at time of first harvest in plots planted with seed stocks that gave various percentages of seed transmission of the virus*

Trial no.	Percentage of plants with lettuce mosaic at time of first harvest in plots planted with seed that gave the indicated percentage seed transmission							
	1.6	0.8	0.4	0.2	0.1	0.05	0.025	0.0
1	27.5	8.9	7.6	4.2	3.6	1.5	1.5	1.4
2	19.4	9.2	8.4	7.1	4.8	2.7	2.6	1.2
3	22.5	15.7	13.2	12.2	11.3	7.8	8.4	4.4
4	14.2	8.0	7.4	3.6	2.6	1.4	2.3	1.3
5	47.5	35.6	34.3	18.2	11.5	8.8	3.9	5.2
6	82.9	75.9	77.7	47.6	23.1	17.3	11.6	8.3
7	8.1	3.2	2.4	2.9	1.0	—[b]	—[b]	0.6
8	10.1	4.5	3.6	2.4	1.0	—[b]	—[b]	0.5
Average[a]	29.5	20.7	20.1	12.5	7.6	6.8	5.5	3.4

[a] These percentages were calculated from the total number of mosaic-affected plants and the corresponding grand total of all plants in all tests at each level of seed transmission.

[b] Not planted.

upon vector prevalence and activity. For example, in trial 7 only 8.1 per cent of the plants were infected in the plot planted with the seed stock that gave 1.6 per cent seed transmission, but 47.5 and 82.9 per cent of the plants in the corresponding plots in trials 5 and 6, respectively, were infected (Table 1). Nevertheless, there was a consistent over-all reduction in total percentage of infection in all trials as the percentage of mosaic from seed-borne virus was reduced from 1.6 per cent to 0.0. In trial 5, for example, 47.5 per cent of the plants in the plot planted with seed that gave 1.6 per cent seed transmission were infected at the time of the third reading, which was made just prior to the first harvest. The other plots in this trial showed a gradual reduction in total percentage of mosaic to as low as 5.2 per cent in the plot planted with virus-free seed. The percentage of mosaic in the 0.025-seed-stock plot in this trial was slightly lower (3.9 per cent) than that in the virus-free-seed plot (5.2 per cent), but this difference is undoubtedly not significant and represents the only instance in which the virus-free-seed plot did not have the lowest percentage of total infection.

Growers and seedsmen have often asked, "How low a percentage of seed transmission is required to give adequate control of mosaic?" This, of course, depends to a large extent upon prevalence and activity of the vectors of the virus, but data on average infection in all 8 trials (Table 1) show that if the percentage seed transmission is above 0.1 per cent, control is likely to be unsatisfactory.

The highest rate of increase in percentage of infected plants occurred during the latter third of the growing season. This was true in all the trials, and the data from trial 6 (Table 2) illustrate this point. In trial 6, for example, the total percentage of infected plants in the plot planted with seed that gave 1.6 per cent seed transmission increased from 5.8 per cent at the time of the first reading to 14.9 per cent by the time of the second reading, which was made 14 days later. During the 22 days that elapsed between the second and third readings, the percentage of plants that became infected increased by 68 per cent to bring the total percentage in the plot to 82.9.

Pattern of spread.—Calculations based on a seeding rate of 1 lb. of seed to the acre and 348,000 seed to the pound show that approximately 250 infected seed were introduced into each of the plots planted with seed that gave 1.6 per cent seed transmission and correspondingly smaller numbers into the plots planted with seed with lower percentages of seed transmission. It would have been extremely difficult to map accurately the position of all the infected plants in the plots prior to thinning. Therefore, the first reading and mapping was made a few days after thinning. Unfortunately, the plants that showed symptoms at the time of the first reading were not necessarily produced from infected seed because it was evident that considerable spread had occurred prior to thinning in several of the trials. Therefore, the only definite information that is known about the plants that showed symptoms at the time of the first reading is that infection resulted either from seed transmission or from spread that had occurred prior

TABLE 2.—*Percentage of lettuce plants with lettuce mosaic at time of first reading and the increase in percentage of diseased plants during 2 subsequent periods in plots planted with seed stocks that gave various amounts of seed transmission of the virus*

Percentage seed transmission by seed stock	Percentage of plants with mosaic at first reading and the increase in this percentage, in 2 subsequent periods, recorded on the indicated date as the increase since the previous reading									Total percentage of plants infected at final reading		
	Trial 1			Trial 5			Trial 6					
	March 26	April 11	May 5	April 9	April 30	May 19	May 9	May 23	June 14	Trial 1	Trial 5	Trial 6
1.6	1.5	3.4	22.6	3.5	6.1	37.9	5.8	9.1	68.0	27.5	47.5	82.9
0.8	1.0	1.6	6.3	2.2	5.0	28.4	4.4	7.1	64.4	8.9	35.6	75.9
0.4	0.5	1.2	5.9	1.9	5.0	27.4	3.8	7.5	66.4	7.6	34.3	77.7
0.2	0.2	0.9	3.1	0.7	1.9	15.6	2.3	3.7	41.6	4.2	18.2	47.6
0.1	0.2	0.7	2.7	0.4	1.2	9.9	1.2	1.3	20.6	3.6	11.5	23.1
0.05	0.0	0.1	1.4	0.3	0.4	8.1	0.8	1.0	15.5	1.5	8.8	17.3
0.025	0.2	0.2	1.1	0.0	0.3	3.6	0.1	0.7	10.8	1.5	3.9	11.6
0.0	0.0	0.3	1.1	0.0	0.2	5.0	0.0	0.3	8.0	1.4	5.2	8.3

to thinning. Because of the negligible amount of early infection that occurred in the virus-free-seed plots, however, it can be assumed that practically all of the very early infection in the other plots originated from seed-borne virus.

The plants that showed mosaic symptoms after the first reading undoubtedly were not produced from infected seed because all such plants would have showed symptoms at the time of the first reading. It can be assumed, therefore, that all of the diseased plants recorded after the first reading represented secondary infections. Approximately 25 per cent of the plants mapped at the second reading were immediately adjacent to plants that showed symptoms at the time of the first reading. It seems likely, therefore, that the inoculum sources for infection of these plants came from the previously infected plants that were adjacent. Approximately 75 per cent of the plants mapped at the second reading occurred at random in the plots with no apparent relationship to plants that showed symptoms at the time of the first reading. The question arises as to the source of inoculum for these plants. There are several possibilities: 1) short random flights of infective aphids within the plot; 2) close association of these plants with those produced from infected seed, or with very early secondarily infected plants that were removed at time of thinning; and/or 3) flights of infective aphids from outside of the plots. This last named possibility can be minimized, or largely disregarded, because of the negligible amount of infection that was apparent at the time of the second reading in the plots planted with virus-free seed. Of the first 2 possibilities listed above, there is no information concerning which was operative. Either one, or both, could explain the apparent random distribution of 75 per cent of the plants that showed symptoms at the time of the second reading.

In all trials, approximately 79 per cent of the late secondary spread (third reading) occurred in definite disease centers in the plots planted with seed that gave 1.6, 0.8, and 0.4 per cent seed transmission. These disease centers were located around plants that had shown symptoms at the first or second readings and extended down the bed, with little tendency to cross the furrow into adjacent beds. This definite tendency of the disease centers to extend down the bed rather than across the furrow suggests that a major portion of the late secondary spread was effected by crawling aphids rather than by aphids making short random flights.

Approximately 21 per cent of the late secondary spread occurred at random in plots planted with seed that gave 1.6, 0.8, and 0.4 per cent seed transmission. Some of this infection can be attributed to viruliferous aphids that came into the plots from the outside; however, the low total percentages of infected plants in the virus-free-seed plots, as a whole, indicated that this mode of infection was of relatively minor importance. It seems more likely, therefore, that most of these scattered infections resulted from short random flights of viruliferous aphids within the plots.

Discussion.—The strong tendency for the late secondary infection to occur in disease centers that extended down the bed rather than across the furrow to adjacent beds is not necessarily in disagreement with the patches of infected plants reported by Broadbent et al[4]; this difference in results probably can be explained by a difference in the 2 planting methods used. In Broadbent's studies, the lettuce was planted on flat ground with the rows 12 in. apart, and the plants were thinned to 10–12 in. in the row. An equal distance separating plants in the row and in adjacent rows would allow crawling aphids to move with equal ease in all directions. In contrast, the lettuce in the present experiments was planted on a raised ridge 20 in. across the top on which 2 rows are planted 14 in. apart. The plants are thinned in the row to intervals of 11–14 in. Under these conditions, the distance between adjacent rows on neighboring beds is 26 in., with a furrow in between. This distance, plus the furrow that is often wet or muddy because of irrigation, may act as a barrier to movement of crawling aphids and thereby restrict spread of the disease; however, further studies on the movements of aphids within the crop will be necessary to confirm the hypothesis that late spread of mosaic is effected to a large extent by viruliferous aphids that crawl from plant to plant.

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[4] Broadbent, L., T. W. Tinsley, W. Buddin, and E. T. Roberts. 1951. The spread of lettuce mosaic in the field. Ann. Appl. Biol. 38: 689-706.

THE INFLUENCE OF HYDROGEN ION CONCENTRATION ON THE EMERGENCE OF GOLDEN NEMATODE LARVAE[1]

Trevor Robinson[2] and A. L. Neal

SUMMARY

The optimum pH for the emergence of golden nematode (*Heterodera rostochiensis* Wr.) larvae was found to be between pH 2.0 and 3.0 for aqueous solutions of hydrochloric, citric, or fumaric acid. When tomato plant leachings were adjusted to pH 2.5 with hydrochloric acid, the emergence of larvae may be stimulated, retarded, or only slightly affected. When leachings diluted 10-fold were tested, there was a stimulation of hatch at pH 2.5. In some experiments, the hatch in the diluted leachings at this lower pH exceeded the hatch from the initial leachings. These findings suggest that anhydrotetronic acid previously reported as an artificial hatching stimulant for the golden nematode may not be a specific hatching stimulant, but rather that its effectiveness may be the result of the effect on the acidity of the hatching medium.

Previous workers who have studied the effects of pH on the emergence of golden nematode (*Heterodera rostochiensis* Wr.) larvae have agreed that pH has no effect over a relatively wide range. Ellenby[3] used a number of different buffer solutions and reported a rapid decrease of emergence below pH 3.4 and no toxic effect if mineral acids were used to adjust the acidity. Fenwick[4] reported no significant effect on emergence between pH 3.2 and 8.0 when hydrochloric acid or sodium carbonate were used to adjust the acidity of potato root-diffusates.

Observations made in our laboratory from time to time indicate that certain erratic results might have been due to stimulation of emergence at low pH values. Therefore, experiments were undertaken to reinvestigate the influence of acidity upon larval emergence.

MATERIALS AND METHODS.—Glass distilled water (25 ml) that had been adjusted to the required pH with hydrochloric acid was added to a Petri dish containing approximately 5 mg of golden nematode cyst material (100–150 cysts). After incubation at 20°C for 1 week, the cysts and larvae were counted. Assays normally were run in duplicate, and the individual values averaged. During incubation, the pH usually increased by about 0.1 unit and never more than 0.3 unit in the range from pH 1.0 to 4.5. Above pH 4.5, there was often a greater increase (0.5 unit); but since no significant effect of pH on emergence was noted at values greater than pH 4.0, no attempt was made to maintain the acidity at a constant value.

In other experiments, tomato (*Lycopersicon esculentum* Mill.) root leachings were adjusted to pH 2.5 with hydrochloric acid.

So-called "normal leachings" were obtained from tomato plants grown in soil in flats with wire mesh bottoms and lined with cheesecloth. These were subjected to a mist of tap water for about 8 hours, and the liquid that drained through the soil was collected. During this period, about 150 liters of leachings were obtained from 300 plants. The pH of these leachings averaged about 7.0.

Assays also were made with cysts that had been ruptured by piercing the cyst membrane with a fine needle. In these experiments, samples of 25 cysts instead of 5 mg were used for each treatment.

RESULTS.—Although the absolute figure for larval emergence per cyst varied among assays for any given treatment, there was unquestionably higher hatching at pH values between 2 and 3 than at values outside of this range (Fig. 1). A test of the significance of treatment means gives $F = 18.7$, which is significant at the 1 per cent point, hence real differences exist among treatment means. In other experiments with citric and fumaric acids, the maximum emergence was within the same pH range, although the absolute stimulation was less than when hydrochloric acid was used. Nitric and oxalic acids at pH 2.5 were inhibitory.

The larvae hatched at pH 2 appeared fully as active as those hatched in distilled water, even when the incubation period was extended to 2 weeks; however, incubation at pH 1 appeared to produce a permanent toxic effect.

The apparent disagreement of the present data with those of Fenwick[4] evidently is not due to the fact that Fenwick used presoaked cysts for his study whereas

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[3] Ellenby, C. 1946. Ecology of the eelworm cyst. Nature [London] 157: 451-452.

[4] Fenwick, D. W. 1951. Investigations on the emergence of larvae from the cysts of the potato root eelworm, Heterodera rostochiensis. 4. Physical conditions and their influence on larval emergence in the laboratory. Jour. Helminth. 25: 37-48.

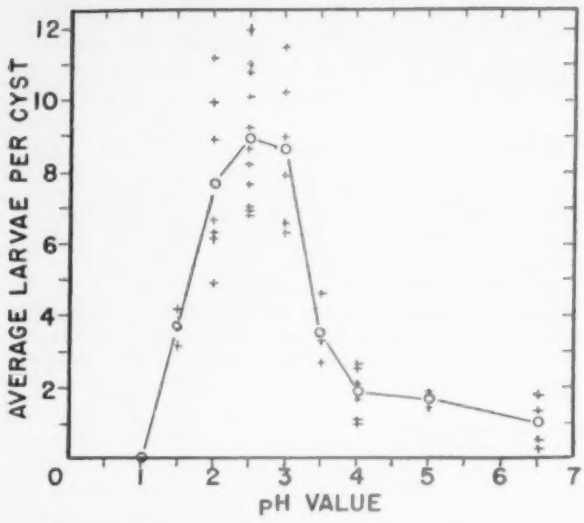

FIG. 1. Emergence of golden nematode larvae in aqueous hydrochloric acid solutions at different pH values. The curve was constructed from the average of the various values obtained. The value at pH 6.5 is the distilled water blank.

the cysts were not presoaked in these tests. Preliminary experiments, not presented in detail here, indicated parallel results when presoaked cysts were used, although the total emergence of larvae was consistently higher than when cysts that were not presoaked were used. The probable cause of the disagreement was that Fenwick used root diffusate rather than distilled water. This possibility was further tested.

Data presented in Table 1 illustrate the variations in hatch observed when the acidity of leachings was adjusted to pH 2.5 with hydrochloric acid. It is apparent that activity is dependent not only upon the pH

TABLE 1.—*Average emergence of golden nematode larvae in distilled water, in tomato-root leachings, and in these media adjusted to pH 2.5*

Assay medium	Average larval emergence[a] per cyst in 3 experiments			
	English cysts		Belgium cysts	
	Leaching A	Leaching B	Leaching A	Leaching B
Normal leachings				
pH 7 (approximate)	12.0	32.5	97.3	53.6
pH 2.5	2.0	3.4	54.4	67.7
Normal leachings diluted 1:10				
pH 7 (approximate)	5.2	7.4	29.2	12.2
pH 2.5	7.9	10.0	135.0	97.7
Distilled water	1.2	1.5	2.0	(2.0)[b]
HCl, pH 2.5	4.8	7.7	29.0	(29.0)[b]

[a] Each figure is the average of duplicate determinations each involving 100–150 cysts.

[b] Data with Belgium cysts were obtained from a single experiment, therefore this value is the same for both batches of leachings.

TABLE 2.—*Emergence of larvae from punctured cysts*

Assay medium	Average larval emergence[a] in 4 experiments			
	A	B	C	D
Distilled water, pH 6.4 (approximate)	3.0	4.7	2.6	2.2
HCl, pH 2.5	9.5	15.4	6.8	8.7
Normal leachings, pH 7 (approximate)		29.4		

[a] Each figure is the average of duplicate determinations, each involving 100–150 cysts.

but also upon the particular cysts used as well as upon the nature of the leachings. In some cases, there was a marked decrease in the stimulatory effect of leachings when they were adjusted to pH 2.5, whereas in other instances only a slight effect was noted. This latter observation is in accord with the data of Fenwick.[4] Furthermore, from the data of 1 experiment it appears that a decided stimulatory effect also may be encountered. Of particular interest are the data obtained from leachings diluted 10-fold. The diluted leachings were always more active at the lower pH, and in some assays the number of larvae that emerged even exceeded that with normal leachings. With normal leachings, the marked stimulatory effect of lowered pH apparently is obscured by the presence of unknown inhibitory substances. With leachings diluted 10-fold, the inhibitory effect of these substances appear to be decreased to such an extent that the stimulatory effect of lowered pH becomes apparent.

As noted with intact cysts, marked stimulation in hatching at pH 2.5 also was observed with ruptured cysts (Table 2), an indication that the stimulatory effect of lowered pH is not necessarily the result of increased permeability of the cyst wall or of the unplugging of the natural openings in the cyst. The permeability of individual egg membranes may also have been changed. Another explanation might be that the hypothetical inhibitor proposed by Ellenby[3] may be inactivated at low pH.

DISCUSSION.—These results on the stimulatory effect of acid solutions at pH 2.0–3.0 on the hatching of golden nematode larvae, suggest that the data obtained by Calam et al[5] on the stimulatory effect of anhydrotetronic acid on the hatching of golden nematode larvae may have resulted, at least in part, from the effect of this agent on acidity of the preparations. This compound was reported to be inactive at pH values greater than 3 and to possess maximum activity at pH 2.65.

It must be emphasized that response to acidity may vary with the particular lot of cysts used. We have

[5] Calam, C. T., A. R. Todd, and W. S. Waring. 1949. The potato eelworm hatching factor. 2. Purification of the factor by alkaloid salt fractionation. Anhydrotetronic acid as an artificial hatching agent. Biochem. Jour. 45: 520-524.

observed stimulation at pH 2.5 with cysts obtained from Belgium, from England, and from Long Island, N. Y. One lot of cysts obtained from England did not respond markedly to pH changes in distilled water, although some slight response was noted when traces of salts were added or when tap water was used as the assay medium. This unresponsive lot of cysts had been stored for over a year, whereas the reponsive cysts were all used within a year of the time they were collected.

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IMROVED CYTOCHEMICAL METHODS FOR DIFFERENTIATING CRONARTIUM RIBICOLA FROM CRONARTIUM OCCIDENTALE ON RIBES[1]

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SUMMARY

On Ribes plants, the uredial and telial stages of the white pine blister rust fungus (*Cronartium ribicola*) are so similar morphologically to those of the pinyon pine blister rust fungus (*C. occidentale*) that it is impractical to distinguish them routinely by their morphology. Two cytochemical methods were developed that distinguish between the telia of *Cronartium ribicola* and those of *C. occidentale* on Ribes. In method 1, the Acree-Goss brom phenol blue indicator solution for staining telial columns was tested at different pH values and was found to give more distinct and reliable results at pH 3.4 than did the original method or the modification described by Cave. In method 2, which may be used in the field, the telial columns were stained with acid fuchsin solution buffered at pH 4.8. Telial columns of *C. ribicola* retained the red stain, but those of *C. occidentale* were rapidly destained by buffer. It is considered that this difference in staining is due to a difference in the isoelectric points of the proteins of the 2 species.

As the white pine blister rust fungus continues to spread in forests of the western states, there is an increasing need for a rapid and dependable method for identifying it in the uredial and telial stages on Ribes species. In some forests of the Pacific Coast, Ribes may be infected by either the white pine blister rust fungus (*Cronartium ribicola* Fisch. v. Waldh.), by the pinyon pine blister rust fungus (*C. occidentale* Hedgc., Bethel, & Hunt), or by both. The former rust fungus is of great economic importance on pines, whereas the latter is relatively unimportant. Consequently, supervisors responsible for the eradication of Ribes as a means of controlling white pine blister rust need rapid methods for distinguishing these 2 species of fungi. The uredia and telia of these fungi are morphologically so similar that their differentiation is difficult.

The most promising methods that have been developed to differentiate these rust fungi have been those that make use of slight differences in the staining reaction of the telial columns of the 2 species. Up to the present time, the cytochemical test described by Acree and Goss[3] and modified by Cave[4] has been the principal procedure used to distinguish between these fungi in the telial stage. This method makes use of 0.001 M brom phenol blue indicator adjusted to pH 7.6 as the test reagent. Properly treated telial columns of *C. ribicola* turn blue when placed in a drop of the indicator; those of *C. occidentale* become green. We have found that the Acree-Goss method does not always clearly distinguish the 2 species, especially when applied to specimens collected on the Pacific Coast. In addition, the test has proved to be somewhat time-consuming and, because of the need for a microscope, is not entirely satisfactory for use in the field. Accordingly, research was initiated in an effort to develop a more satisfactory procedure. Various histochemical methods, involving stains, indicators, and other reagents were tried; as a result, 2 satisfactory methods have been developed

[1]Accepted for publication July 16, 1956.
[2]This project was conducted in cooperation with the United States Forest Service. The Methods Development Unit, Blister Rust Control, supplied the infected Ribes used in this work and provided financial assistance for rust identification work; the California Forest and Range Experiment Station provided a grant-in-aid for the research.

[3]Acree, R. J., and W. H. Goss. 1937. A microchemical colorimetric pH procedure for differentiating the telia of Cronartium ribicola and C. occidentale. Jour. Agr. Res. 55:347-352.

[4]Cave, M. S. 1944. Modification of procedure for differentiating the telia of Cronartium ribicola and C. occidentale. Stain Technol. 19:141-142.

for identifying the 2 rust fungi in the telial stage.

BROM PHENOL BLUE METHOD.—This method is a modification of the Acree-Goss brom phenol blue procedure. The indicator was prepared by adding 5 mg of merthiolate to 100 ml of a 0.001 M aqueous solution of brom phenol blue and adjusting the mixture to pH 3.4 by adding 0.1 N NaOH solution. A drop of the indicator solution was placed on a microscope slide, and to this were added 8–10 telial columns that had been removed from a lesion by means of sharp-pointed jeweler's tweezers. This was done under a dissecting microscope. A cover glass (No. 2, 22-mm diameter) was added and pressed gently to insure contact of the stain with the telial columns. The slide was placed in a saturated atmosphere in a petri dish for 5–6 minutes to allow penetration by the indicator. The telial columns were examined under the low power of a compound microscope; curved-tipped forceps were used to press the cover slip until the color of the individual teliospores was clearly visible. The telial columns of *C. ribicola* stained blue; those of *C. occidentale* varied from orange-red through red to purple. After 10–15 minutes in the indicator, the columns of both species became dull green, and identification became impossible. Since a compound microscope is required, this method is not as satisfactory as the acid fuchsin method for use in the field.

When pressure was applied to the cover glass during the preparation of mounts for the brom phenol blue method, it was found that the columns of *C. ribicola* remained somewhat flattened after slight pressure but did not break up. Those of *C. occidentale* appeared to be much more resilient and tended to retain their original form.

The blue color of *C. ribicola* corresponded to the color of the brom phenol blue indicator at pH 4.3 and above[5], whereas the orange-red color shown by *C. occidentale* appeared similar to the color of the indicator at approximately pH 3.7. When both rust fungi were treated with 4 other indicators that showed strong color changes between pH 3.5 and 4.5, however, no indication of distinct difference in the internal pH of the 2 fungi was obtained. It therefore appears that factors other than pH must be concerned in this test.

ACID FUCHSIN METHOD.—This method is essentially the method used by Tarassevich[6] to obtain evidence regarding isoelectric points of virus proteins. It was assumed that the proteins of different rust fungi, like viruses, might have different isoelectric points and therefore might stain differently at certain pH values. It was found that an acid fuchsin solution buffered at pH 4.8 gave the greatest difference in staining of the 2 fungi.

The buffer solution was prepared by mixing 50 ml of M/5 potassium acid phthalate ($KHC_8H_4O_4$) and 17.7 ml of M/5 NaOH and diluting to 200 ml. The pH was tested with a pH meter and adjusted to 4.8[3]. As a preservative, 5 mg of merthiolate was added to each 100 ml of buffer. If desired, the buffer solution may be purchased from a chemical supply house. The staining solution was a 0.5 per cent solution of acid fuchsin in the above buffer.

A drop of the staining solution was placed on a microscope slide. Under a hand lens or a dissecting microscope, 8–10 telial columns were removed from a dried Ribes leaf and placed in the drop of stain. A cover glass was applied and the columns allowed to stain for 5 minutes. Drops of buffer were then added to one edge of the cover grass while the staining solution was drawn from the opposite edge by means of filter paper. When removal of the free stain appeared complete, the slide was placed in a saturated atmosphere in a petri dish for 5–6 minutes to complete the removal of free stain. The mounts were examined under a hand lens or a dissecting microscope; the telial columns of *C. ribicola* remained a deep red color, whereas those of *C. occidentale* destained slowly in the buffer and gradually resumed their natural light brown color.

When fresh undried leaves were used as a source of the fungi, the stain did not penetrate the telial columns satisfactorily. When infected portions of such leaves were held in 70 per cent alcohol for 15 minutes or longer before removal of the telial columns, penetration by the stain was satisfactory.

During our experimental work, acid fuchsin solutions buffered at different pH values were tested. It was found that both species stained most strongly at pH 3, the lowest tested. As the pH was increased, the intensity of the staining decreased; however, at pH 4.8 *C. ribicola* still stained heavily, whereas *C. occidentale* retained much less stain. At higher pH values, both species stained less intensely as the pH was increased. This behavior was in accordance with the expected staining of proteins and apparently indicated that the proteins of the telial columns of *C. ribicola* have a higher isoelectric point than do those of *C. occidentale*. At pH 4.8, the protein of *C. ribicola* apparently is positively charged and therefore retains the negatively charged acid fuchsin. In contrast, the protein of *C. occidentale* apparently is neutral or negatively charged at this pH and does not retain the negatively charged dye. It is hoped that this principle may be applied in distinguishing between other fungi that are not readily identifiable by morphology.

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[5]Clark, W. M. 1928. The determination of hydrogen ions. Williams and Wilkins, Baltimore. 717 p.

[6]Tarassevich, L. M. 1945. Determination of isoelectric point of virus proteins by staining techniques. Acad. des Sci. U. R. S. S. Comp. Rend. (Dok.) 47:666-668.

COMPARATIVE RESISTANCE OF SEVEN PEPPER VARIETIES TO FIVE ROOT-KNOT NEMATODES [1]

W. W. Hare [2]

SUMMARY

The reaction of 7 selected pepper varieties to 5 different root-knot nematodes was tested. With the rating system used, Early California Wonder, Oakview Wonder, and 4795B Peru were susceptible to *Meloidogyne incognita acrita* and *M. incognita*. Early California Wonder was moderately resistant to *M. javanica* and susceptible to *M. arenaria*, whereas Oakview Wonder and 4795B Peru were resistant to both species. Red Chili and P. I. 135824 were moderately resistant to *M. incognita acrita* and *M. incognita* and resistant to *M. javanica* and *M. arenaria*. *Santanka* xS and 405B Mexico were resistant to all species except *M. hapla*. All varieties were susceptible to *M. hapla*.

Differences in reaction of pepper varieties (*Capsicum frutescens L.*) to a root-knot nematode later identified as *Meloidogyne incognita acrita* Chitwood were found earlier (5). Attention was directed to the fact that the genus *Meloidogyne* (formerly *Heterodera marioni* (Cornu) Goody) had been divided into several species in 1949 (3), about the time the pepper work was initiated. Work was then carried out to determine how the pattern of varietal reaction to the one nematode compared to that to other common forms of root-knot nematodes described as occurring in the United States.[3] An abstract has been published (4).

No account of testing the reaction of pepper to specific nematodes has been seen by the author other than that of Sasser (6), who tested 1 variety to 5 species and a second variety to 1 species. The nematode species concerned cannot be determined in reports of tests made earlier than 1949. Since that date there have been numerous reports of this type of work on other crops with a single variety or collection, e.g. Taylor and Chitwood (7), or a range of varieties or strains, e.g. Allison (1).

METHODS AND MATERIALS.—Seven varieties or strains of pepper, 405B Mexico, Santanka xS, Red Chili, P.I. 135824, 4795B Peru, Oakview Wonder, and Early California Wonder, were used in the tests. Stock cultures of the root-knot nematodes *M. incognita* (Kofoid & White) Chitwood, *M. incognita acrita* Chitwood, *M. javanica* (Treub) Chitwood, *M. arenaria* (Neal) Chitwood, and *M. hapla* Chitwood were maintained on Southland tomatoes. These cultures originated from single egg masses and were increased on tomato. A sixth nematode culture was obtained by taking soil from a greenhouse bench where previous tests with pepper had been conducted. After galls developed on tomatoes in this soil, the culture was handled in the same manner as the other 5 cultures. This "native" culture had been identified as *M. incognita acrita* (5).

Hardware cloth and glass wool were placed in the bottoms of 8-in. pots, 1 in. of sand was added, and the remaining space was filled with potting soil. After a pot and its contents were autoclaved for 1 hour at 15 lb. pressure, the top layer of soil was removed and chopped galls from tomato roots grown on the proper culture were spread over the surface. One-half of the soil was replaced, and the pepper seed were planted and covered with the remaining soil. When susceptible varieties had well-developed galls, the individual plants were removed and rated for reaction to the nematodes. Root-knot indices were prepared as described earlier (5).

There was some variation in the survival of seedlings from the different seed lots but none in seedlings grown in soil infested with the different nematode cultures except for the native culture, where survival was significantly lower. Evidently a damping-off fungus was brought over in the mass soil-transfer. The average number of plants per index calculated was 9, 13, and 15 for tests 1, 2, and 3, respectively. Notes were taken after 77, 89, and 81 days' growth in the 3 tests.

RESULTS.—In the first test, 6 of the varieties or strains were used; in previous work (5), 2 of these varieties had been rated healthy to slight, 2 slight to moderate, and 2 severe in reaction to *M. incognita acrita*. In terms of resistance, these varieties were considered resistant, moderately resistant, and susceptible, respectively. The root-knot indices obtained are shown in Table 1. For the purpose of classifying varieties on the basis of resistance, those with indices of zero to 25 were grouped as resistant; 26–40, moderately resistant; and 41–75, susceptible.

The indices of the varieties from soil infested with *M. incognita acrita* were similar to the earlier results (5). Those resistant to *M. incognita acrita*, 405B Mexico and Santanka xS, were also resistant to *M. incognita*, *M. javanica*, and *M. arenaria* but were susceptible to *M. hapla* (Fig. 1, A, B). Those moderately resistant to *M. incognita acrita*, P.I. 135824 and Red Chili, were the same to *M. incognita*, susceptible to *M. hapla*, and resistant to *M. javanica* and *M. aren-*

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TABLE 1.—*Root-knot indices of peppers grown in soil infested with Meloidogyne spp. (root-knot nematodes)*

Nematode species	Test No.	Indices[a] of indicated variety or strain of pepper[b]						
		Oakview Wonder	4795B Peru	Early Cal. Wonder	P.I. 135824	Red Chili	Santanka xS	405B Mexico
M. incognita acrita (native)	1	69.4	70.0		37.5	27.5	25.0	15.0
	2			75.0		25.0		31.3
	3			75.0		18.2		6.3
	Average	69.4	70.0	75.0	37.5	23.6	25.0	17.5
M. incognita acrita	1	71.4	62.5		35.7	30.6	5.0	0
	2			75.0		28.3		20.3
	3			75.0		37.5		0
	Average	71.4	62.5	75.0	35.7	32.1	5.0	6.8
M. incognita	1	75.0	75.0		30.0	37.5	0	0
	2			75.0		25.0		0
	3			75.0		46.7		0
	Average	75.0	75.0	75.0	30.0	36.4	0	0
M. javanica	1	0	0		0	0	0	0
	2			18.1		0		0
	3			58.3		0		0
	Average	0	0	38.2	0	0	0	0
M. arenaria	1	25.0	25.0		25.0	0	0	0
	2			23.3		0		0
	3			69.2		0		0
	Average	25.0	25.0	46.3	25.0	0	0	0
M. hapla	1	75.0	75.0		75.0	42.5	56.3	60.7
	2			67.6		50.0		62.5
	3			75.0		58.9		64.3
	Average	75.0	75.0	71.8	75.0	50.5	56.3	62.5

[a] The number of plants in each root-knot class was multiplied by the class value (healthy, 0; slight, 25; moderate, 50; and severe, 75), the totals added, and the sum divided by the total number of plants to obtain the root-knot index for each entry.

[b] Oakview Wonder, Early California Wonder, and Red Chili were from commercial seed sources, and 405B Mexico from Dr. P. G. Smith, University of California. Dr. H. H. Foster obtained 4795B Peru and Santanka xS from Dr. W. H. Greenleaf, Georgia Experiment Station, Experiment, Georgia, and P.I. 135824 from Dr. W. S. Porte, U. S. Department of Agriculture, Beltsville, Maryland.

aria. With *M. arenaria* and *M. hapla*, Red Chili had lower indices than P.I. 135824, although both varieties fell into the same resistance grouping. Oakview Wonder and 4795B Peru were susceptible to all the nematode species except *M. javanica* and *M. arenaria*. They were resistant to the last 2 species, but there was a distinct difference in the indices; indices of both varieties were zero with *M. javanica* and 25 with *M. arenaria*.

Only 1 variety representing each classification to *M. incognita acrita*, resistant, moderately resistant, and susceptible, was used in the second and third tests. An error in seed selection resulted in the use of Early California Wonder as the susceptible variety in the second test. This variety was continued in the third test, since it appeared to be more susceptible to *M. javanica* and *M. arenaria* than was Oakview Wonder or 4795B Peru. This strain of Early California Wonder was the only pepper on which *M. javanica* produced galls (Fig. 1, C). Otherwise, results of the second test were essentially similar to those of the first (Table 1) with the exception of the 20.3 index of 405B Mexico grown in soil infested with *M. incognita acrita*. Results of the third test were also in agreement with those of the first 2 tests (Table 1). As controls, Southland tomatoes were grown in all 3 of the tests in soil infested with each nematode culture, and the tomato and all pepper varieties used in the particular test were grown in clean soil. All tomato plants grown on nematode cultures were severely galled at the times notes were taken, and there were no galls on roots of noninoculated tomato and pepper plants.

When it was noted that Early California Wonder appeared to be more susceptible to *M. javanica* and *M. arenaria* than were Oakview Wonder and 4795B Peru, possible contamination by other nematodes was checked by examination of specimens from roots of pepper grown in soil containing the respective nematode cultures. Identifications of the nematodes agreed with the labels. This difference in varietal reaction was selected for further verification from among several in the results that were similar. Oakview Wonder and Early California Wonder were grown in 3 replicates; half of each variety was inoculated with *M. javanica* and the other half with *M. arenaria*. An average of 16 plants was classified for each index prepared. With *M. javanica*, Oakview Wonder had indices of 0, 0, and 0 in the 3 replicates, and Early California Wonder had indices of 39.1, 34.2, and 38.9 (average, 37.4). With *M. arenaria*, Oakview Wonder had indices of 23.6, 25.0, and 21.4 (average, 23.3), and Early California Wonder had indices of 29.2, 51.8, and 40.6 (average, 40.5). The average indices agreed

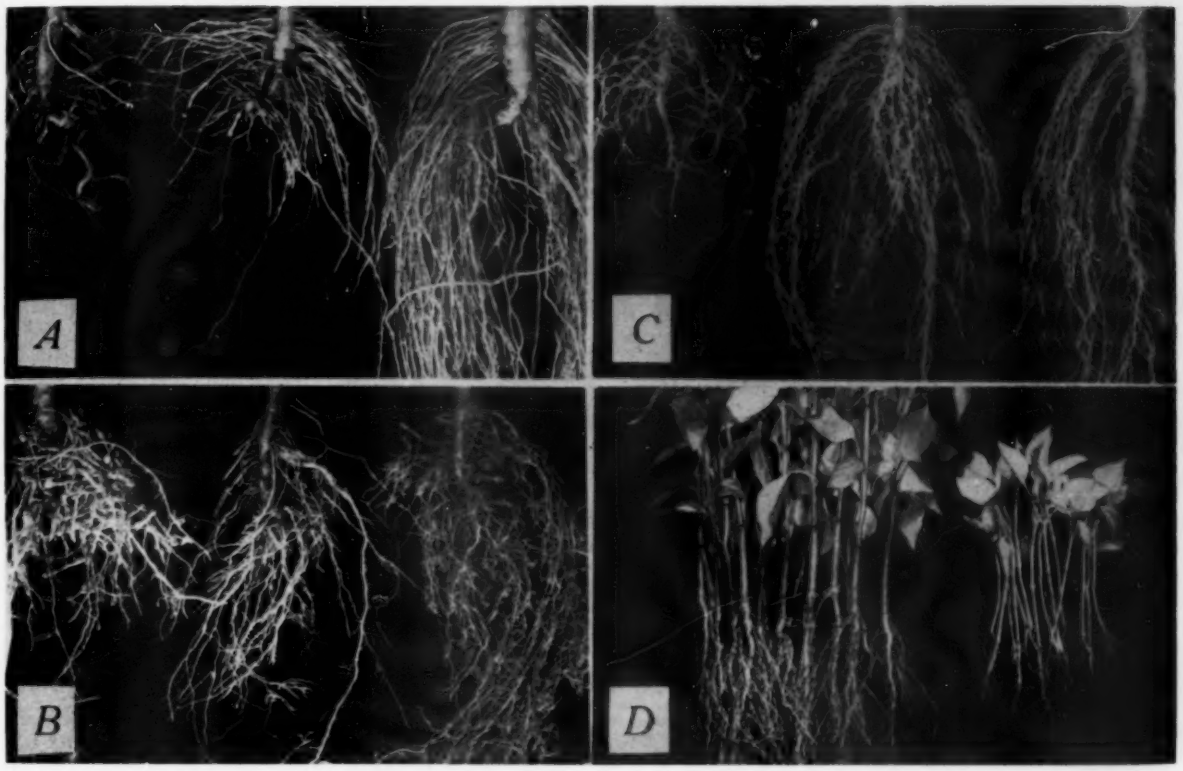

FIG. 1. A, B) Left to right: Oakview Wonder, Red Chili, and 405B Mexico pepper grown 77 days in soil infested with A) *Meloidogyne incognita* and B) *M. hapla*. C) Left to right: Early California Wonder, Red Chili, and 405B Mexico grown 89 days in soil infested with *M. javanica*. D) 405B Mexico, left, and Early California Wonder, right, grown 118 days in soil infested with *M. incognita acrita*.

closely with those already obtained.

The index of 405B Mexico grown in soil infested with *M. incognita acrita* was zero in 2 tests and 20.3 in the other (Table 1). This variability in reaction of 405B Mexico, and of other varieties possessing similar resistance, to that nematode was observed in previous work. When galls were produced, they tended to be smaller on 405B Mexico than on susceptible varieties and to grow from the roots somewhat like bacterial nodules on legumes. Some factors were noted that appeared to influence this attack on the resistant varieties, although no single factor could be clearly demonstrated in experiments set up for that purpose. They were 1) continued growth of successive crops on the same nematode culture, 2) slow growth of the pepper plants, 3) both susceptible and resistant plants grown in the same pot, and 4) increased length of growing period. Early California Wonder and 405B Mexico were grown in soil infested with *M. incognita acrita* for 118 days, with all the favorable conditions provided in accordance with the observations made. Indices of Early California Wonder from all pots were 75.0, and those of 405B Mexico varied from 19.1 to 75.0. A group of plants of 405B Mexico that were rated severe on the basis of class definition are shown in Figure 1, D along with Early California Wonder grown in the same pots. The 405B Mexico plants had well-developed root systems and vigorous tops of about the same size as non-inoculated controls.

DISCUSSION.—Two of the nematodes worked with here, *M. javanica* and *M. arenaria*, affected pepper not at all or so lightly that they could be disregarded as economic factors except on occasional pepper strains such as the Early California Wonder. Although there were varietal differences in reaction to *M. hapla*, no resistance was found that was considered enough to warrant attempts to transfer it to other varieties.

The widest differences in resistance of the several pepper strains were found among those to *M. incognita* and *M. incognita acrita*, probably the most widely distributed and damaging of the root-knot nematodes in agricultural soils of the southern states. Varying degrees of resistance were found in small-fruited hot peppers but none in bell or pimento types. Varieties appear to behave alike in reaction to each of the 2 nematodes, with the exception of the variable amount of galling incited by *M. incognita acrita* on the most resistant group of varieties. This galling appeared to be atypical and some conditions were observed that influenced it, though no single one could be clearly demonstrated. It has been pointed out (2, 6) that "resistance" to root-knot nematodes is resistance to the production of galls and reproduction of the

nematodes rather than to entry of the nematodes into the tissues. In the present case, the term "resistance" may be overworked when applied to a variety when plants are grown under certain conditions and rated severe in reaction on the basis of the presence and amount of galls on the roots. There was no question, however, about the difference between the condition of the "resistant" plants and that of those called "susceptible" (Fig. 1, D). Since there appeared to be a complete range of indices from zero to 75, only 3 terms were used ("resistant," "moderately resistant," and "susceptible"), and these were applied to the groups of varieties on the basis of their indices when grown under comparable conditions. "Tolerance" was not introduced, because so little was understood about the described type of attack by *M. incognita acrita* on 405B Mexico.

No clear line could be drawn between differences in indices that indicated real varietal differences in reaction to nematodes and those that did not. There was great variation in some cases from test to test in the index obtained for a variety on a given nematode. Most of this was ascribed to failure to reproduce test conditions and some of it to possible population variation, since none of the varieties had been pure lined. One of the lesser differences was substantiated in replicated trials, and differences of that order elsewhere, when indices were from the same test or averages of several tests, were considered real; e.g. Red Chili was more resistant than P.I. 135824 to *M. hapla*, but 405B Mexico may or may not have been more resistant than P.I. 135824.

The differences encountered here with a few varieties tested to 5 nematode species suggest that there were several different genes, or sets of genes, in operation to account for them. In all probability, more would be found in more extensive testing of varieties. Prospects for breeding to transfer more of the resistance to commercial bell varieties will be rather dim if many genes have to be moved from the undesirable plant types. Selection of the commercial as well as the resistant parent on the basis of reaction to the common root-knot nematodes may reduce the size of the problem.

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LITERATURE CITED

1. Allison, J. L. 1956. Root knot of perennial forage legumes. (Abs.) Phytopathology **46**:6.
2. Barrons, K. C. 1939. Studies of the nature of root knot resistance. Jour. Agr. Res. **58**:263-272.
3. Chitwood, B. G. 1949. Root-knot nematodes—Part I. A revision of the genus Meloidogyne Goeldi, 1887. Helminth. Soc. Wash. Proc. **16**:90-104.
4. Hare, W. W. 1953. Nematode resistance in pepper. (Abs.) Phytopathology **43**:474.
5. Hare, W. W. 1956. Resistance in pepper to Meloidogyne incognita acrita. Phytopathology **46**:98-104.
6. Sasser, J. N. 1954. Identification and host-parasite relationships of certain root-knot nematodes (Meloidogyne spp.) Md. Agr. Exp. Sta. Tech. Bul. A-77, 31 p.
7. Taylor, A. L., and B. G. Chitwood. 1951. Root knot susceptibility of Lycopersicon peruvianum. U. S. Dept. Agr. Pl. Dis. Rptr. **35**: 97.

PLANT VIRUS INFECTION IN RELATION TO THE INTERVAL BETWEEN WOUNDING AND INOCULATION [1]

Henryk Jedlinski [2]

SUMMARY

When the leaves of the host plants were wounded by rubbing and subsequently inoculated by immersion in virus suspensions, the amount of infection as measured by the number of lesions that developed was usually dependent upon the length of the time interval between injury and inoculation. During the first 10 minutes, susceptibility increased, decreased, or remained constant, depending upon the host-virus combination used. The previously reported rapid decrease in the susceptibility to tobacco mosaic virus of injured leaves of *Nicotiana glutinosa* was confirmed; a very great decrease occurred within the first minute. Similar results were obtained with tobacco mosaic virus and *N. tabacum* var. Java Isere × Cabot. Susceptibility of injured leaves of *N. rustica* to tobacco mosaic virus decreased during the first 30 seconds but later increased to a high level. Leaves of *N. rustica* reacted in the same maner to tobacco necrosis virus, but the subsequent increase was much greater. In the case of tobacco necrosis virus and *Vigna sinensis*, the susceptibility increased for 5 minutes, then decreased. With the increase of the interval between the injury and inoculation, the susceptibility of *V. sinensis* to tobacco ringspot virus increased for 5 minutes and then remained unchanged for 5 minutes; that of *Phaseolus vulgaris* increased slowly but steadily during the 10 minutes. The length of the interval tested had little or no effect on infection of *P. vulgaris* by tobacco mosaic virus or of *N. tabacum* by tobacco necrosis virus. The observed increases of susceptibility during the time after wounding appear not to be in agreement with the theory that the virus infection consists primarily of an introduction of virus particles into slightly wounded cells; instead, they appear to favor an absorption theory based on one that has been outlined for bacterial and animal viruses.

The processes involved in the infection of plants by viruses are still obscure, and the explanations given by various investigators are conflicting to a large extent. Local-lesion counts, the method commonly used in studies of infection phenomena, *per se*, do not distinguish factors governing successful virus establishment in the host from subsequent multiplication. It may be quite correct to assume that the active virus becomes successfully established only on, or in, cells that under suitable conditions are capable of supporting the virus multiplication. On the contrary, the factors governing the virus establishment that permits subsequent multiplication may be entirely different from those controlling multiplication. This differentiation is apparent with some bacterial and animal viruses which have been investigated more successfully (14).

When leaves of *Nicotiana glutinosa* L. are injured by rubbing and then sprayed with suspensions of tobacco mosaic virus, the number of infections decreases rapidly as the interval between injury and inoculation is increased (1, 9, 16). Within 1–3 hours, leaves recover from injuries to such an extent that no infection results after inoculation (9, 10). These observations apparently led a number of investigators to speculate that a successful establishment of infection depends primarily on the introduction of a plant virus particle into a slightly wounded cell (2, 4, 15). Kalmus and Kassanis (9) present the view that leaves injured with abrasives cannot repair in a short time the damage to the cuticular layer to prevent the entry of salts, but can restore "semipermeable systems" that prevent entry of large particles such as viruses.

Direct methods in studies of plant virus infection have not proved to be very successful, in part because of the lack of information on the processes involved in the initiation of the infection. Microchemical analysis (7), use of electron microscopy (18), and local-lesion methods (19) have failed to detect virus multiplication within 8–60 hours following inoculation.

The same problem has been approached by various indirect methods. Many workers have attempted to elucidate the character of the quantitative response of the host to various doses of virus. Statistical methods have been applied in a measurement of the dilution effect on the number of local lesions produced by different plant viruses (2, 6, 11, 12, 17, 20, 21). In those studies where the main attention was focused on the number of virus particles necessary for infection, different workers reached different conclusions: 1) that local infection develops as a result of introduction into susceptible host-tissue of at least 1 virus particle (2, 12, 20) and that there is a finite number of uniformly susceptible entry points in any 1 experiment (12, 20); and 2) that individual susceptible regions vary in susceptibility so that different numbers of virus particles are necessary to pro-

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duce infection at different sites (11).

The effect of nutrition on the infection processes was reviewed by Allington and Laird (1), who investigated the effect of potassium nutrition on the susceptibility of *N. glutinosa* to infection with tobacco mosaic virus. It was found that in low-potassium plants, the receptive condition of wounded leaf areas to the virus persisted longer and at a much higher level than in the plants grown under normal nutrient supply. According to these authors, this increase in susceptibility emphasizes the "dynamic nature" of the receptive sites and the importance of potassium in the processes of infection.

The present study was concerned with the investigation of the susceptibility of certain plants to some viruses at different time intervals after wounding. Of particular interest was the problem of whether the previously reported results with *N. glutinosa* and tobacco mosaic virus are typical of other virus-host combinations. The results from this study throw further light on the processes involved in the establishment of infection.

MATERIALS AND METHODS.—The level of susceptibility of the plants to the viruses at different time intervals after wounding was measured by the local-lesion methods. The following species of *Nicotiana* were used: *N. glutinosa*, *N. rustica* L., and *N. tabacum* L. var. Java Isere × Cabot, which gives a local-lesion reaction with tobacco mosaic virus like that reported by Holmes (8). The above plants were started in small flats in composted soil. At the 2- to 3-leaf stage, they were transplanted into 5-in. pots and kept on the sand bench in the greenhouse with the temperature maintained at 23°C ± 5°. When the 7- to 10-leaf stage was reached, they were used in the experiment. Seeds of *Phaseolus vulgaris* L. var. Scotia and of *Vigna sinensis* Endl. var. Black Eye were germinated in "rag dolls" in an incubator and then transplanted into 4-in. pots. These plants were kept on a sand bench at 26° C ± 5°. The primary leaves only were utilized in the experiment. In the fall and winter, a 16-hour day length was maintained by supplemental light from 500-watt incandescent bulbs. The viruses used were those of common tobacco mosaic, yellow strain of common tobacco mosaic, tobacco necrosis, and tobacco ringspot.

The virus cultures were maintained in plants of *N. tabacum* var. Havana No. 38. Tobacco mosaic virus was partially purified by the method of Commoner (7), whereas yellow tobacco mosaic virus and tobacco necrosis virus were partially purified by differential centrifugation at approximately 4000 G and 78,500 G for 40 minutes and 1½ hours, respectively. The virus pellets were suspended in 0.1 M buffer prepared from sodium acetate and acetic acid adjusted to pH 5.7 and stored in the refrigerator at 5° C. The inocula used were prepared by appropriate dilutions of stock preparations with distilled water at the time of the experiment. Uniform inoculum was always used within each experiment. Because of instability of tobacco ringspot virus, diluted crude sap of diseased plants was used as inoculum in experiments with this virus. Symptomatic leaves of *N. tabacum* were harvested 2–3 weeks after inoculation and ground in a domestic meat mincer. The sap then was pressed out through 4 layers of fine cheesecloth, diluted 1:10 with distilled water in a room at 21° C ± 5°, and used immediately at a temperature of 21° C ± 2°.

Leaves of *Nicotiana* species were inoculated and incubated as described by Allington and Laird (1). The plants were selected for uniformity and were dusted with 600-mesh Carborundum. Then the leaves were rubbed with dry fingers, and at the desired time intervals they were removed individually from the plants by breaking them off at the base of the petiole. They were submerged instantaneously in the inoculum and then rinsed by a second dip in distilled water, without further wounding. The inoculated leaves subsequently were placed on wire screens in shallow pans with the petioles submerged in tap water and incubated in a moist chamber under 40-watt fluorescent lights at 18° C ± 3° and a relative humidity near 90 per cent. Under these conditions, local lesions developed after 3 or 4 days; final readings were recorded at this time. Similar methods were used for leguminous plants except that the 2 primary leaves of 1 plant received the same treatment, and each leaf was submerged separately in the inoculum and subsequently in the distilled water without removal from the plant. This was done by holding the tilted pot containing the plant at the level of inoculum in one hand and pressing down on the stem with the other, causing the leaf to be submerged. The plants were incubated in humid glass chambers partially shaded and located in the greenhouse. Final readings were taken after 4 or 5 days. Because of the conflicting data in the literature on the effect of time of the day of inoculation on the production of local lesions (3, 13), all inoculations were made at the same time of day, in late afternoon.

The different treatments were distributed over the test plants in a latin square design in the case of the *Nicotiana* species. The incomplete block design was used with the leguminous hosts, and each treatment was applied to 5 plants, each with 2 primary leaves. The number of replications was the same as the number of treatments. The treatments represented the time intervals in seconds between wounding and inoculation and were as follows: 0, 30, 60, 300, and 600 seconds. The treatment designated as "0 seconds" represents the shortest interval attainable under the mechanical limitations existing.

EXPERIMENTAL RESULTS.—The following experimental results are concerned primarily with the changes in receptivity of wounded leaves of various species to different viruses during the 10 minutes immediately following the wounding. The degree of receptivity is expressed by the number of local lesions produced. The terms receptivity and susceptibility are used interchangeably in these studies to connote the tendency for a successful establishment between

the host and virus of a relationship that permits subsequent virus multiplication. The variability in the number of local lesions produced among experiments was within a satisfactory range unless otherwise stated.

Each susceptibility curve shown in Figure 1, A is based on data averaged from 3–5 experiments. In these tests with yellow tobacco mosaic virus, the different hosts reacted quite differently. Receptivity of wounded leaves of *N. glutinosa* and *N. tabacum* var. Java Isere × Cabot decreased rapidly during the first minute and then remained at a low level. In the case of *N. rustica*, receptivity decreased slightly for the first 30 seconds and then increased to a level higher than that immediately following wounding.

In tests with a common strain of tobacco mosaic virus, the receptivity of *P. vulgaris* remained approximately at a constant level for the time tested.

The changes in receptivity to tobacco necrosis virus of 4 different hosts (following wounding) were entirely different from those described for *N. glutinosa* and *N. tabacum* var. Java Isere × Cabot to yellow tobacco mosaic virus. Each susceptibility curve shown in Figure 1, B is based on data averaged from 4–6 experiments. Susceptibility of wounded leaves of *N. rustica*, *V. sinensis*, and *P. vulgaris* to tobacco necrosis virus increased at different rates during the first 5 minutes after wounding, except for a small decrease during the first 30 seconds in the case of *N. rustica*. In contrast, the receptivity of *N. tabacum* var. Java Isere × Cabot remaind at a relatively constant level for the 10 minutes.

The results with tobacco ringspot virus on *V. sinensis* and *P. vulgaris* (Fig. 1, C), comprising data averaged from 4 experiments each, also differ from those obtained with *N. glutinosa* and yellow tobacco mosaic virus (Fig. 1, A). The receptivity of *V. sinensis* increased very rapidly in the first 5 minutes after wounding and did not change during the next 5 minutes. In the case of *P. vulgaris*, there was a steady increase in receptivity for the entire 10 minutes.

Discussion.—The results of this work further illustrate the complexity of the processes involved in virus establishment. The susceptibility curves (Fig. 1, A, B, C) point out the dynamic nature of the processes involved in infection. These processes appear to be specific in respect to the host and to the infecting virus. No generalization can be made about the nature of the processes involved in establishment of the infection from the reaction of 1 virus on 1 host. Thus the reaction of *N. glutinosa* to tobacco mosaic virus as described by Sheffield (16) and Kalmus and Kassanis (9) is not necessarily typical of all cases. Furthermore, Allington and Laird (1) have shown that plants of a given species when grown at a low potassium level may remain receptive to this virus for a longer period of time and at a higher level than will normal plants. This increase in receptivity appears from the present studies to be

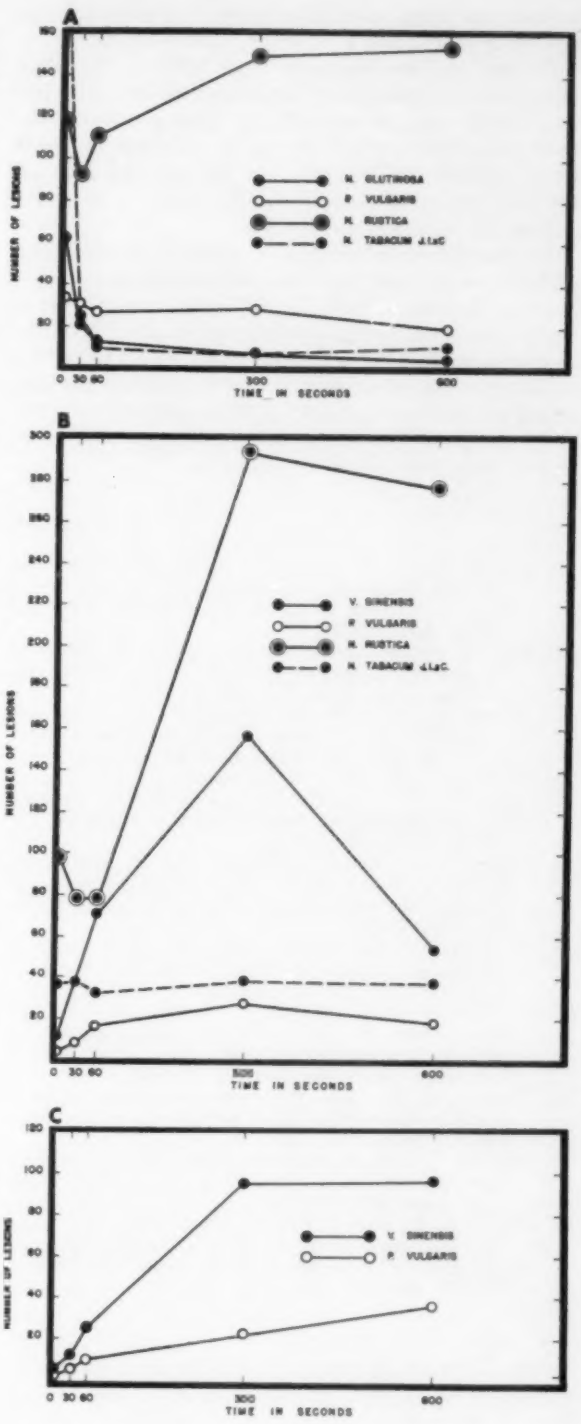

Fig. 1. Average numbers of local lesions that developed on leaves that were injured by rubbing and subsequently inoculated at given time intervals. A) Tobacco mosaic virus (a common strain was used on *Phaseolus vulgaris*, and yellow tobacco mosaic on the other 3 hosts). B) Tobacco necrosis virus. C) Tobacco ringspot virus.

normal and even more accentuated in certain other host-virus combinations.

No one has demonstrated what part of the plant virus particle, commonly considered as the infective unit, enters the living cell. It appears highly unreasonable that permeability of a wounded cell to a virus particle can increase for 10 minutes and yet the cell can recover or heal sufficiently to allow for subsequent virus multiplication.

In view of these findings it appears doubtful that infection of a plant host results from the mere introduction of virus particles into slightly wounded cells. The explanation of the mechanism of plant virus infection suggested by Allington and Laird (1), which is based on an adsorption theory as outlined for bacterial and animal viruses by Puck and Sagik (14) and includes the possibility that nonwounded cells may be involved, becomes more plausible.

At the present time one can only speculate as to the nature of the processes involved in the successful virus establishment in the host cell. In this respect, we may interpret the results of these studies to indicate that there are certain conditions necessary for both the virus and the host to initiate and promote this reaction, which appears to be specific for given host-virus combinations. No explanation for the observed phenomena is offered. Certain physical and chemical processes depending on the time factor may be responsible for the period of optimum receptivity of the host to the virus after wounding.

The value of assays would be greatly enhanced by taking into consideration the most receptive time of the indicator plant after wounding.

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LITERATURE CITED

1. Allington, W. B., and E. F. Laird, Jr. 1954. The infection of Nicotiana glutinosa with tobacco mosaic virus as affected by potassium nutrition. Phytopathology **44**: 297-299.
2. Bald, J. G. 1937. The use of numbers of infections for comparing the concentration of plant virus suspensions. Ann. Appl. Biol. **24**: 33-52.
3. Bawden, F. C., and F. M. Roberts. 1948. Photosynthesis and predisposition of plants to infection with certain viruses. Ann. Appl. Biol. **35**: 418-428.
4. Bawden, F. C. 1950. Virus diseases and host-plant physiology. In his Plant viruses and virus diseases. Ed. 3. Chronica Botanica, Waltham, Mass. p. 278-279.
5. Bawden, F. C., and G. O. Freeman. 1952. The nature and behavior of inhibitors of plant viruses produced by Trichothecium roseum Link. Jour. Gen. Microbiol. **7**: 154-168.
6. Caldwell, J. 1936. Factors affecting the formation of local lesions by tobacco mosaic virus. Roy. Soc. London, Proc., Ser. B. **119**: 493-506.
7. Commoner, B., F. Mercer, P. Merrill, and A. Zimmer. 1950. Microanalytical determination of the rate of tobacco mosaic virus synthesis in tobacco leaf tissue. Arch. Biochem. **27**: 271-286.
8. Holmes, F. O. 1953. The tendency of some types of tobacco to escape infection by tobacco-mosaic virus. Phytopathology **43**: 475.
9. Kalmus, H., and B. Kassanis. 1945. The use of abrasives in the transmission of plant viruses. Ann. Appl. Biol. **32**: 230-234.
10. Kassanis, B., and A. Kleczkowski. 1948. The isolation and some properties of a virus-inhibiting protein from Phytolacca esculenta. Jour. Gen. Microbiol. **2**: 143-153.
11. Kleczkowski, A. 1950. Interpreting relationships between concentrations of plant viruses and numbers of local lesions. Jour. Gen. Microbiol. **4**: 53-69.
12. Lauffer, M. A., and W. C. Price. 1945. Infection by viruses. Arch. Biochem. **8**: 449-468.
13. Matthews, R. E. F. 1953. Factors affecting the production of local lesions by plant viruses. I. The effect of time of day of inoculation. Ann. Appl. Biol. **40**: 377-388.
14. Puck, T., and Sagik, B. 1953. Virus and cell interaction with ion exchangers. Jour. Exp. Med. **97**: 807-820.
15. Sadasivan, T. S. 1940. A quantitative study of the interaction of viruses in plants. Ann. Appl. Biol. **27**: 359-367.
16. Sheffield, F. M. L. 1936. The susceptibility of the plant cell to virus disease. Ann. Appl. Biol. **23**: 498-505.
17. Spencer, E. L., and W. C. Price. 1943. The accuracy of local lesion methods for measuring virus activity. Amer. Jour. Bot. **30**: 280-290.
18. Steere, R. L. 1952. Virus increment curves obtained from counts of particles in clarified plant juice. Amer. Jour. Bot. **39**: 211-220.
19. Yarwood, C. E. 1952. Latent period and generation time for two plant viruses. Amer. Jour. Bot. **39**: 613-618.
20. Youden, W. J., H. P. Beale, and J. D. Guthrie. 1935. Relation of virus concentration to the numbers of lesions produced. Boyce Thompson Inst. Contrib. **7**: 37-53.
21. Youden, W. J. 1937. Use of incomplete block replications in estimating tobacco mosaic virus. Boyce Thompson Inst. Contrib. **9**: 41-48.

SURVIVAL OF STREPTOMYCES SCABIES IN PEAT SOIL PLANTED WITH VARIOUS CROPS [1]

W. J. Hooker [2]

SUMMARY

Peat soil heavily infested with *Streptomyces scabies* (Thaxt.) Waks. & Henrici was used to fill concrete bins that were 5 ft. × 6 ft. and 2½ ft. deep. These bins were located in a general farming area 25 miles from potato fields. Scab was uniformly severe on a crop of Cobbler potatoes grown the first year. In subsequent years, different plots were planted to corn, to onions, to soybeans, to potatoes, or left fallow. Soil samples were removed each spring for plate counts of *Streptomyces* spp. and for growing Cobbler potato plants in the greenhouse to determine scab incidence on the tubers produced. Deep (3 and 4 type) scab on tubers of the Cobbler variety was only slightly less following the second year of fallow or of planting to either onions or corn than on tubers grown in soil from either the potato or soybean plots. From the third to the sixth year, incidence of deep scab on tubers grown in soil from onion, corn, soybean, or fallow plots was distinctly lower than that from the potato plots. Scab incidence was influenced to a greater extent by the length of time between potato crops than by any of the 4 crops tested. Trends were similar with shallow (1 and 2 type) scab although differences were not as well defined. Plate counts of streptomycetes isolated from the soil during the 6-year period were highest in the corn plots, almost as high in the potato plots, and slightly lower in the onion and soybean plots. These counts were consistently lowest in the fallow plots. It was not possible to correlate plate counts of *Streptomyces* spp. with scab incidence in these trials.

The influence of cropping practice on scab incidence in subsequent crops of potatoes is incompletely understood. Lutman et al (13) reported that pathogenic strains of *Streptomyces scabies* (Thaxt.) Waks. & Henrici died out during 19 years between potato crops in 1 of his field tests. In trials involving typical crop rotation studies under irrigation in Nebraska (3), scab was most severe in short potato rotations and in those involving sugar beets. Scab was least severe in 4- to 6-year rotations with alfalfa. Goss and Livingston (4) reported that the crop preceding potatoes in 5-year dry land rotations (corn, wheat, or summer fallow) and in 3-year rotations (corn, wheat, or beans) was not as important as the length of the rotation. They postulated that the influence of the preceding crop might be greater under conditions of soil moisture levels higher than those present in their plots. Werner, Kiesselbach, and Goss (21) observed during a 10-year period that there were no significant differences in scab incidence due to the crop preceding potatoes when the rotations were of equal length. There was no real advantage to a 5-year rotation over a 3-year rotation. Blodgett (1) reported that scab incidence was highest where potatoes were grown every year as contrasted to 2- or 5-year rotations. There was no clear-cut case for the preference of any 1 crop in the rotation for the reduction of scab. Wheeler (22) reported that scab incidence in a rotation consisting of a potato, corn-seeded-to-rye-in-last-cultivation, potato sequence was approximately equal to that in a potato, oats-seeded-to-alfalfa, alfalfa, alfalfa, potato sequence. Scab incidence in a rotation with a potato, oats and sweet clover, potato sequence was much higher than in either of the other 2 rotations and also higher than in continuous cropping to potatoes.

A distinction should be drawn between results reporting scab incidence in rotation experiments in which mature plant debris has decomposed in the soil and results from experiments involving green manuring for scab control. Millard (15) demonstrated excellent scab control in greenhouse trials involving green manure, and later Millard and Taylor (16) attributed control of this type to antagonism between *S. scabies* and *S. praecox* (Millard & Burr.) Waks. & Henrici. Sanford (18) reported no scab control following application of 50 tons of green rye per acre. Later (19), reduction of scab in nonsterilized artificially infested soil was obtained when either green rye or green clover was incorporated in the soil. Control was attributed to microbial antagonism. In greenhouse trials, scab incidence (17) was lowest following green manuring with soybean vines, considerably higher following green manuring with either red clover or with rye, and slightly higher still in soil continuously cropped to potatoes. In contrast to these results, KenKnight (9) obtained an increase in scab severity following the addition of 20 tons of either green alfalfa or of bluegrass to potato soil.

In laboratory tests of a number of plants involving root inoculation with *S. scabies*, onions and corn were very resistant to cortical root necrosis, whereas soybean and potato were susceptible under the experimental conditions employed (5). The investigations

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reported herein were designed in part to determine the relationship existing between results from such laboratory trials and those from tests in the field.

As far as the author is aware, no reports are available concerning influences of rotation practices on scab incidence on potatoes grown in peat soils. This is a report of work carried out in peat soils in northern Iowa. Scab incidence and populations of *Streptomyces* spp. were determined in peat soils continuously cropped to plants other than potatoes over a period of 5 years. An abstract of this work has been published (6).

MATERIALS AND METHODS.—A calcareous, reed and sedge peat (pH 6.5–7.0) heavily infested with *S. scabies* was trucked from Crystal Lake, Iowa, into a general farming area at Kanawha, Iowa. The latter is located approximately 25 miles from commercial potato fields. Concrete bins that were 5 ft. × 6 ft. and 2½ ft. deep were filled to within 6 in. of the top, and 3 replications were prepared. Within each replication, 5 treatments consisted of fallow (clean cultivation), continuous cropping to potato, to onion, to soybean, and to corn (Fig. 1). These concrete bins were constructed to avoid the contamination between plots that is common in conventional rotation experiments because of machinery movement and surface drainage water.

Precautions were taken to prevent contamination from bin to bin during cultivation and weeding operations. Garden tools were dipped in copper sulfate solution before being used on the next crop, and the operator did not walk in the soil of these bins. The soil was of good fertility at the initiation of the trial, and no fertilizer was added during the course of the investigation. In the fall, all plant parts, including the portions normally harvested, were returned to the soil and buried at approximately a 6-in. depth.

During the first year (1949), a crop of Cobbler potatoes was grown in all bins in order to determine the distribution of *S. scabies* and to increase the amount of such inoculum. Scab was uniformly severe

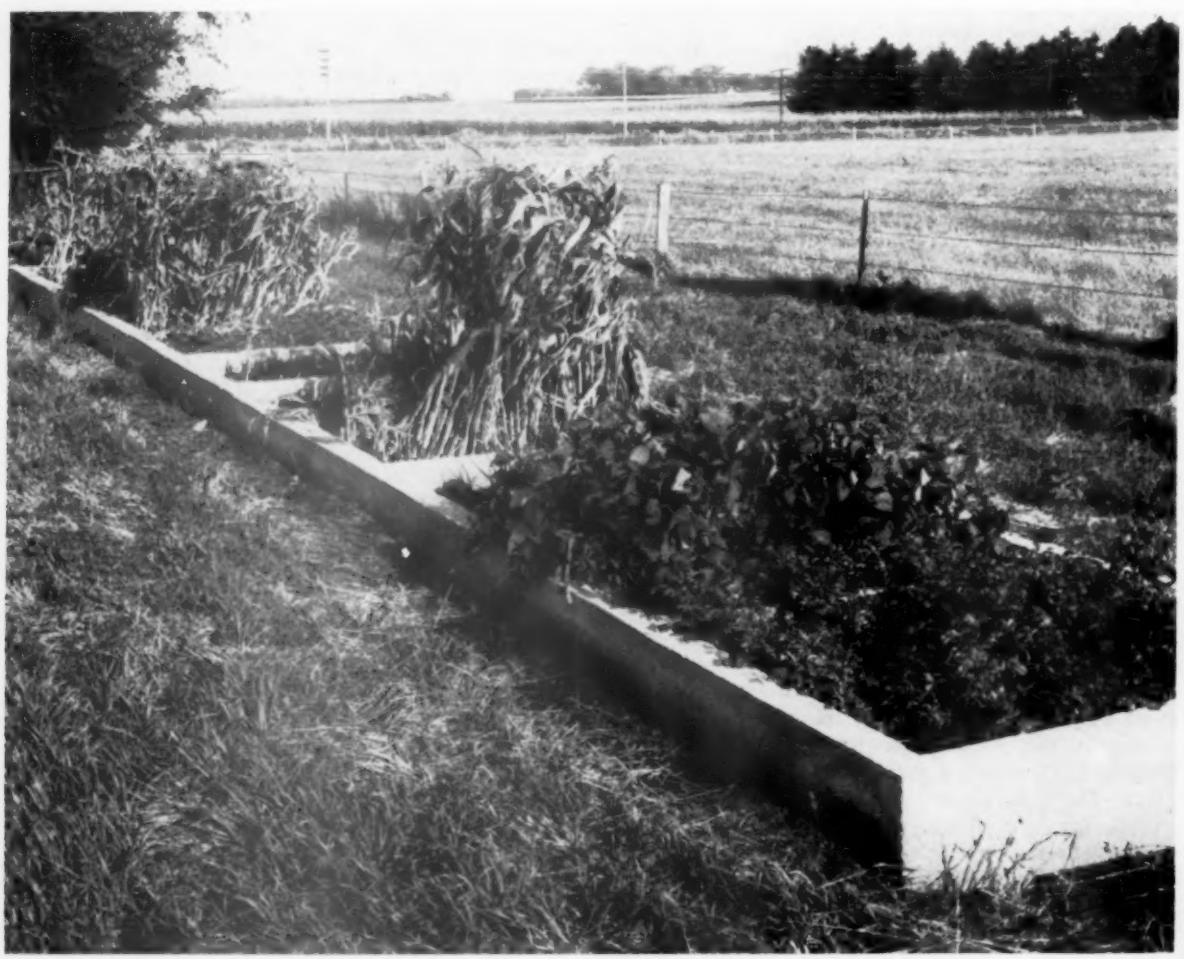

FIG. 1. General view of the concrete bins containing peat soil that were used for determining the influence of various crops on incidence of potato scab.

TABLE 1.—*Populations of Streptomyces spp. in peat soil planted to various crops*

Crop	Populations[a] of *Streptomyces* spp. by years following last potato crop					
	1950 (2 years)	1951 (3 years)	1952 (4 years)	1953 (5 years)	1954 (6 years)	Average[b]
Potato	14178	4250	166	1501	1801	4379
Onion	5253	3239	118	1615	1740	2393
Corn	14444	6013	258	2360	2383	5092
Soybean	8274	4203	167	1211	2106	3192
None (fallow)	4715	2877	86	1017	825	1904

[a] Each figure in these columns represents number of colonies in thousands per g dry soil when plated on soil agar. Each figure is based on at least 9 plates. Three plates were prepared from each of 3 replications. Soil heavily infested with *S. scabies* in concrete bins was cropped to Cobbler potatoes in 1948 and continuously planted to indicated crop.
[b] Least significant differences of averages are 1032 at the 5 per cent level and 1376 at the 1 per cent level.

at the end of the season. Scab incidence on tubers from the various bins was approximately equal, suggesting that inoculum was distributed evenly among the plots.

Each spring, soil samples were removed from each plot and portions used for determining populations of *Streptomyces* spp. by the dilution plate technique. Other portions of each sample were used for growing potato plants (*Solanum tuberosum* L. var. Cobbler) in the greenhouse.

POPULATIONS OF STREPTOMYCES SPP. IN PEAT SOIL.—A number of media reported to be useful in isolating *Streptomyces* spp. from soil were compared for relative efficiency in tests with mixtures of nonsterilized peat soil and known cultures of *S. scabies*. Soil agar as described by Hooker (5) containing the water-soluble material from 25 g of peat soil per liter of 2 per cent agar was the best medium for this use. Colonies of *Streptomyces* spp. were identified easily despite the facts that they were rather restricted in size and growth was sparse. There was practically no contamination by bacteria or fungi on this medium. The medium described by Lutman (12) prepared without calcium carbonate but with 2 per cent agar was somewhat less satisfactory since fungi occasionally overran the plates. Other media tested were Jensen's agar (7) adjusted to pH 7.0, KenKnight and Muncie's agar (10) adjusted to pH 7.0, Waksman's agar (4), Conn's glycerol asparaginate agar adjusted to pH 7.0 (20), and Ashby's mannitol agar as modified by Jones (8). Although these media stimulated luxuriant growth of *Streptomyces* spp., plates often were lost because of overgrowth by filamentous fungi. Soil agar was finally selected for use in these trials.

Methods essentially similar to those used by Goss (2) were modified for this study. Samples of peat soil were sifted through a sterile screen and 10 g were suspended in 200 ml of sterile distilled water contained in a 500-ml Erlenmeyer flask. This was shaken on a wrist-action shaker for 30 minutes, and 5 minutes was allowed for settling of the larger soil particles. Samples for dilution plates were taken from approximately the 1.5-cm depth. Colony counts of *Streptomyces* spp. were determined at dilutions of 1–2000, 1–20,000, and 1–100,000 (Table 1). Populations of *Streptomyces* spp. are expressed as thousands of colonies per gram of soil dried to constant weight at 80°C. Each figure represents combined data from at least 9 plates, 3 plates from each of 3 replications. Data from this randomized block design over the 5 years of observation were treated statistically by the analysis of variance.[3] From 1950 to 1954 inclusive, the moisture content of soil samples ranged from 47 to 56 per cent. In a given year, moisture percentages were very similar in soil from the several plots.

Counts of *Streptomyces* spp. were consistently highest in the corn plots, but the 5-year mean for such plots was not significantly higher than for the potato plots. The mean populations in onion and soybean plots were not statistically different from each other. Populations in onion and fallow plots were significantly lower (1 per cent level) than those from corn and potato plots. Populations in the fallow plots were consistently lower than from any of the other plots.

SCAB INCIDENCE ON TUBERS.—Cobbler potatoes were grown in samples of soil from each of the bins every season and grown directly in the bins the last year of the trial. Seed tubers for all trials were disinfected by the hot-formaldehyde method (14). The tubers produced were rated for scab incidence by determining the percentage of tubers free of scab and the percentage of tubers approximating a satisfactory market grade in which no more than 5 per cent of the surface had deep (types 3 and 4) (11) scab. Disease records were not taken the first year (1949) in which these various crops had been planted to the plots, since it seemed probable that few, if any, differences in scab incidence would be apparent in so short a time. In 1950, the second season, there were slight differences both in the percentage of scab-free tubers (Fig. 2, A) and in the percentage of tubers that approximated a market grade (Fig. 2, B). Although differences were slight, scab was most severe on tubers grown in soil from the potato and soybean plots. From the third to the sixth year, scab generally was severe on tubers grown in soil continuously cropped to potatoes. There were marked differences in scab incidence on tubers produced in such soil as compared to those produced in soil that had been planted with the other crops. There were no consistent differences in the amount of scab

[3] The writer expresses sincere appreciation to Dr. S. K. Ries of the Horticulture Department, Michigan State University, for advice in the statistical treatment of the data.

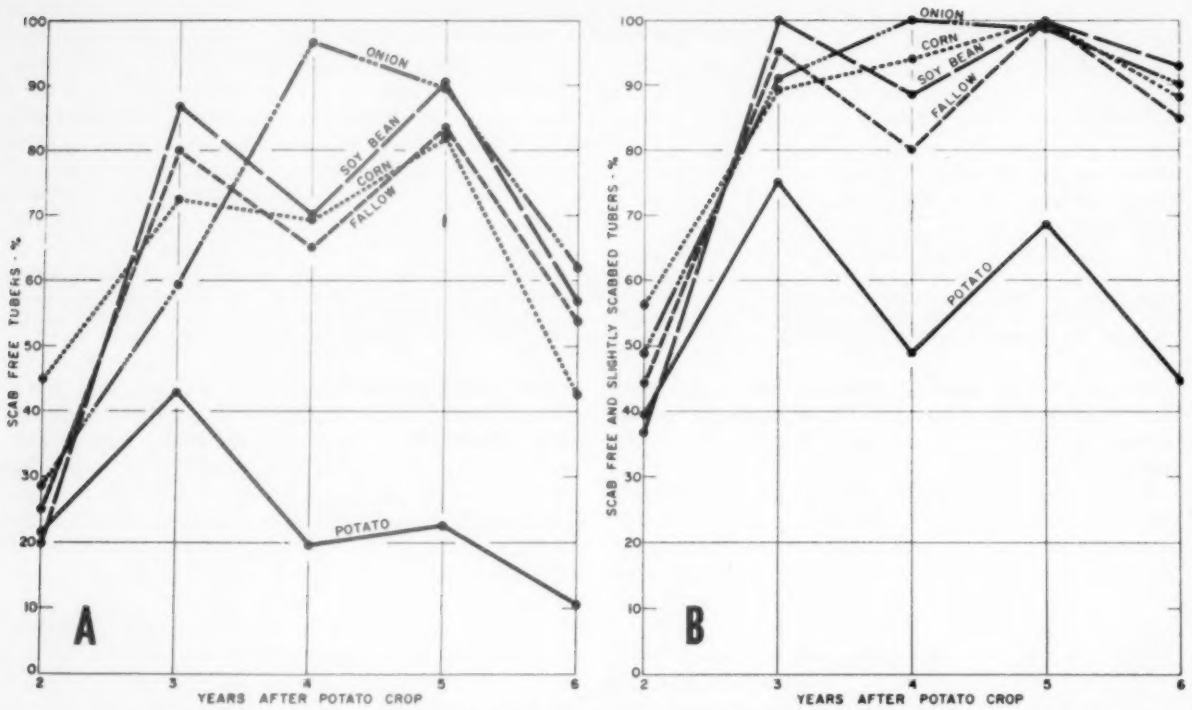

FIG. 2. Percentage of A) scab-free tubers and of B) scab-free plus nearly scab-free tubers grown in peat soil heavily infested initially with *S. scabies* and in subsequent years continuously planted to various crops. In the second to fifth years, tubers were grown in the greenhouse in samples of soil from the plots; in the sixth year, they were grown directly in the plots.

on tubers grown in soil from the onion, corn, soybean, and fallow plots. It appeared that the crop involved was of less importance than the length of time between potato crops. In 1954, Cobbler potatoes were planted in each bin, providing rather large numbers of tubers for the scab records. Scab incidence was somewhat higher under these conditions of growth than in the small plots planted in the greenhouse. Major differences among plots were unchanged. Records were also taken on the incidence of shallow scab (types 1 and 2) (11) on these tubers. Scab of this type was slightly more prevalent on tubers grown in the continuous-potato plots than on those grown in the other plots. There were only slight differences in prevalence of shallow scab on tubers grown in soil from plots other than potatoes and trends were not consistent.

DISCUSSION.—Results of these tests in peat soil in small concrete bins agree rather closely with results reported from field rotation experiments on other kinds of soil in Nebraska (3, 4, 21) and elsewhere (13, 1). The time factor was of greater importance on scab incidence than was the influence of either onion, corn, or soybean when grown between potato crops. None of the crops other than potato influenced scab incidence to a very great extent. The amount of crop debris returned to the soil in these trials was somewhat greater than that normally returned in usual cropping practice. This was due in part to more closely spacing of plants than is customary and in part to the fact that all plant parts were returned to the soil. No green manure as such was added: every season, plant parts were killed by frost before being incorporated into the soil. The 1 possible exception was the incorporation of unfrozen potato tubers. It should be pointed out, however, that these tubers were frozen before spring. There was no evidence to suggest that either onion, corn, or soybean debris stimulated a microflora antagonistic to *S. scabies*, since scab incidence following these crops was very similar to that from the fallow plots.

There was no apparent association between populations of *S. scabies* as measured by scab incidence on tubers and populations of *Streptomyces* spp. in the soil as determined by dilution-plate methods. In the potato, as compared to the corn plots, populations of *S. scabies* were widely different yet populations of *Streptomyces* spp. were very similar. The greatest differences in populations of *Streptomyces* spp. existed between the corn and fallow plots yet there were no measurable differences in populations of *S. scabies*.

There was no apparent correlation between results of laboratory tests of root susceptibility of a number of plants to *S. scabies* (5) and those of these trials in concrete bins. In laboratory tests, potatoes were susceptible to root necrosis. *S. scabies* survived in these peat soil plots in sufficiently heavy populations to scab tubers severely. In contrast, soybean roots, susceptible in laboratory trials, and corn and onion roots, resistant in laboratory trials (5), did not support populations of *S. scabies* sufficient to scab tubers

appreciably. Populations of *S. scabies* were very similar either in fallow soil or in soil planted either to resistant or to susceptible hosts other than potato.

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LITERATURE CITED

1. BLODGETT, F. M. 1940. A second report on the effect of agronomic practices on the incidence of Rhizoctonia and scab of potatoes. Amer. Potato Jour. **17**: 290-295.
2. GOSS, R. W. 1937. The influence of various soil factors upon potato scab caused by Actinomyces scabies. Nebr. Agr. Exp. Sta. Res. Bul. 93, 40 p.
3. GOSS, R. W., AND M. M. AFANASIEV. 1938. Influence of rotations under irrigation on potato scab, Rhizoctonia, and Fusarium wilt. Nebr. Agr. Exp. Sta. Bul., 317, 18 p.
4. GOSS, R. W., AND J. E. LIVINGSTON. 1941. The influence of crop rotations on the occurrence of scab, Rhizoctonia, and Fusarium wilt in potatoes under dry-land conditions in western Nebraska. Nebr. Potato Impr. Assoc. Ann. Rpt. (1940/41) **22**: 22-27.
5. HOOKER, W. J. 1955. Parasitic action of Streptomyces scabies on roots of seedlings. Phytopathology **39**: 442-462.
6. HOOKER, W. J. 1955. Survival of Streptomyces scabies in peat soil planted with various crops. (Abs.) Amer. Potato Jour. **32**: 389.
7. JENSEN, H. L. 1934. Contributions to the microbiology of Australian soils. 1. Numbers of microorganisms in soil and their relation to certain factors. Linn. Soc. N.S. Wales. Proc. **59**: 101-117.
8. JONES, D. H. 1913. A morphological and cultural study of some Azotobacter. Centbl. f. Bakt. [etc.] Abt. II. **33**: 14-25.
9. KENKNIGHT, G. 1941. Studies on soil actinomycetes in relation to potato scab and its control. Mich. Agr. Exp. Sta. Tech. Bul. 178, 48 p.
10. KENKNIGHT, G., AND J. H. MUNCIE. 1939. Isolation of phytopathogenic Actinomycetes. Phytopathology **29**: 1000-1001.
11. LEACH, J. G., F. A. KRANTZ, P. DECKER, AND H. MATTSON. 1938. The measurement and inheritance of scab resistance in selfed and hybrid progenies of potatoes. Jour. Agr. Res. **56**: 843-854.
12. LUTMAN, B. F. 1945. The spread of potato scab in soil by potato plant humus. Vt. Agr. Exp. Sta. Bul. 528, 40 p.
13. LUTMAN, B. F., R. J. LIVINGSTON, AND ALICE M. SCHMIDT. 1936. Soil Actinomyces and potato scab. Vt. Agr. Exp. Sta. Bul. 401, 32 p.
14. MELHUS, I. E., AND G. C. KENT. 1948. Elements of plant pathology. Macmillan Co., New York. 493 p.
15. MILLARD, W. A. 1923. Common scab of potatoes, part II. Ann. Appl. Biol. **10**: 70-88.
16. MILLARD, W. A., AND C. B. TAYLOR. 1927. Antagonism of microorganisms as the controlling factor in the inhibition of scab by green-manuring. Ann. Appl. Biol. **14**: 202-216.
17. ROUATT, J. W., AND R. G. ATKINSON. 1950. The effect of the incorporation of certain cover crops on the microbiological balance of potato scab infested soil. Canad. Jour. Res. Sect. C **28**: 140-152.
18. SANFORD, G. B. 1926. Some factors affecting the pathogenicity of Actinomyces scabies. Phytopathology **16**: 525-547.
19. SANFORD, G. B. 1946. Soil-borne diseases in relation to the microflora associated with various crops and soil amendments. Soil Sci. 61: 9-21.
20. SKINNER, C. E., C. W. EMMONS, AND H. M. TSUCHIYA. 1947. Henrici's Molds, yeasts, and actinomycetes. Ed. 2. Wiley, New York. 409 p.
21. WERNER, H. O., T. A. KIESSELBACH, AND R. W. GOSS. 1944. Dry-land crop rotation experiments with potatoes in northwestern Nebraska. Nebr. Agr. Exp. Sta. Bul. 363, 43 p.
22. WHEELER, E. J. 1946. The residual effect of crop rotations on potato yield and the presence of potato scab. Mich. Agr. Exp. Sta. Quar. Bul. 28. p. 1-7.

LISTING OF BOOKS

PHYTOPATHOLOGY will, from time to time as space becomes available, list titles of books on pathological subjects. A brief description of the contents of the books will be included.

HORSFALL, J. G., JR. 1956. **Principles of fungicidal action.** Chronica Botanica Co., Waltham, Massachusetts; Hafner Publishing Co., New York. Price $6.50.

Whereas the primary emphasis in the author's earlier book on fungicides was on the dosage-response curve as a tool to investigate fungitoxicity, in this new book it is on the mechanism of toxicity and on the types of compounds that are toxic to fungi. The author strives to distinguish, at all points, between permeation and inherent toxicity. The physics and chemistry of protection and the mechanism of fungitoxicity that underlie successful protection are described. The book is prefaced by photostats of extracts from several articles of historical interest and concludes with an extensive bibliography.

DE ONG, E. R. 1956. **Chemistry and uses of pesticides.** Ed. 2 Reinhold Publishing Corp., New York; Chapman and Hall, Ltd., London. Price $8.75.

This is a completely revised edition containing information on insecticides, fungicides, herbicides, rodenticides, repellents, and seed disinfectants. Data are provided on the chemical and physical properties, toxicology, and compatibility of such compounds. Almost half the book is devoted to detailed instruction on the use of synthetic compounds. The appendix contains a dictionary listing 500 compounds, a glossary, a list of tolerances for pesticide residues in or on fresh fruits and vegetables, and a listing of official antidotes.

WHEAT SPOT MOSAIC, CAUSED BY A MITE-TRANSMITTED VIRUS ASSOCIATED WITH WHEAT STREAK MOSAIC [1]

John T. Slykhuis [2]

SUMMARY

Wheat spot mosaic virus frequently occurs together with wheat streak mosaic virus in southern Alberta. It causes chlorotic spots, chlorosis, and stunting, and sometimes kills wheat plants. The virus is transmitted by the eriophyid mite, *Aceria tulipae* Keifer. It was not transmitted by inoculating healthy plants with sap from diseased ones.

All stages of *A. tulipae* except the egg can carry the virus. Susceptible hosts include *Eragrostis cilianensis* (All.) Lutati., *Hordeum vulgare* L., *Setaria italica* (L.) Beauv., *S. verticillata* (L.) Beauv., *S. viridis* (L.) Beauv., *Triticum aestivum* L., *T. dicoccum Schrank*, *T. durum* Desf., *T. timopheevi* Zhukov, and *Zea mays* L. Several Triticum × Agropyron hybrids, immune from wheat streak mosaic virus, are susceptible to spot mosaic virus, but they are difficult to infect and show only mild symptoms.

The eriophyid mite *Aceria tulipae* Keifer is a vector of the wheat streak mosaic virus, and it sometimes carries another factor that causes chlorosis, necrosis, and stunting of wheat in southern Alberta[3]. This factor, unlike wheat streak mosaic virus, has not been transmitted by rubbing expressed sap from diseased plants onto healthy ones, even when Carborundum was added to the sap. The characteristics of transmission indicate that it is a virus. Since a chlorotic spotting is the most consistent early symptom produced on wheat following the feeding of various infective cultures of mites, the disease has been designated "wheat spot mosaic."

EXPERIMENTAL METHODS.—Spot mosaic virus was isolated most frequently from selected wheat plants that showed a severely chlorotic mottle in the field. Mites were transferred individually from these plants to seedlings of winter wheat, *Triticum aestivum* L., var. Kharkov 22 MC, grown singly in sterilized soil in 2.2 × 20-cm test tubes. In 3–8 days most of these plants showed a chlorotic mottle that could be attributed to streak mosaic virus alone, to spot mosaic virus alone, or to a combination of the two. It was necessary to test each chlorotic plant for the presence of streak mosaic virus by mechanical inoculations to healthy plants. Severely chlorotic plants from which streak mosaic could not be isolated were considered to be infected with spot mosaic virus (Fig. 1). Mites that had been reared from the original one placed on these plants were transferred to healthy wheat seedlings, and the virus was perpetuated with the mites.

Occasionally, plants were found with an extreme yellow chlorosis resembling the advanced stages of severe streak mosaic; but streak mosaic virus could not be isolated from them by manual methods. Mites found on these mottled plants were transferred to healthy young plants on which there appeared spot mosaic symptoms. One lot of mites found on an unidentified species of *Agropyron* growing near mosaic-affected wheat transmitted spot mosaic virus but not streak mosaic virus.

Eggs from mites on diseased wheat plants previously were shown to produce noninfective mites[3]. Noninfective colonies of mites were therefore started by hatching eggs obtained either from field- or greenhouse-grown wheat on healthy wheat seedlings growing in sterilized soil in large test tubes. The eggs hatched, and the mites reproduced satisfactorily under these conditions and were very abundant in 4–6 weeks. Mites propagated from these sources were used as noninfective controls in all experiments.

Cultures of noninfective mites and also cultures of mites carrying various isolates of spot mosaic virus were maintained on Kharkov 22 MC winter wheat grown in sterilized soil. The plants were caged under 10 cm × 25-cm cylindrical cages, the bodies of which

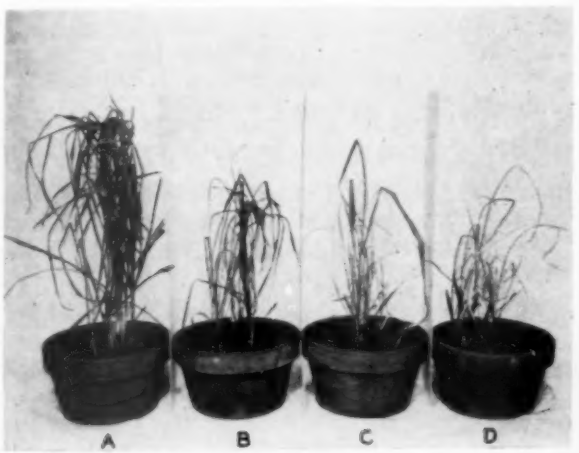

FIG. 1. Kharkov 22 MC winter wheat infested with A) noninfective mites, B) mites that acquired wheat streak mosaic virus from manually inoculated wheat, C) mites carrying spot mosaic virus only, and D) mites cultured from naturally diseased wheat infected with both streak and spot mosaic viruses.

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Contribution No. 1542 from the Botany and Plant Pathology Division, Science Service, Canada Department of Agriculture, Ottawa, Ontario.

[2] Plant Pathologist, Plant Pathology Laboratory, Lethbridge, Alberta, Canada.

[3] Slykhuis, J. T. 1955. Aceria tulipae Keifer (Acarina: Eriophyidae) in relation to the spread of wheat streak mosaic. Phytopathology 45: 116-128.

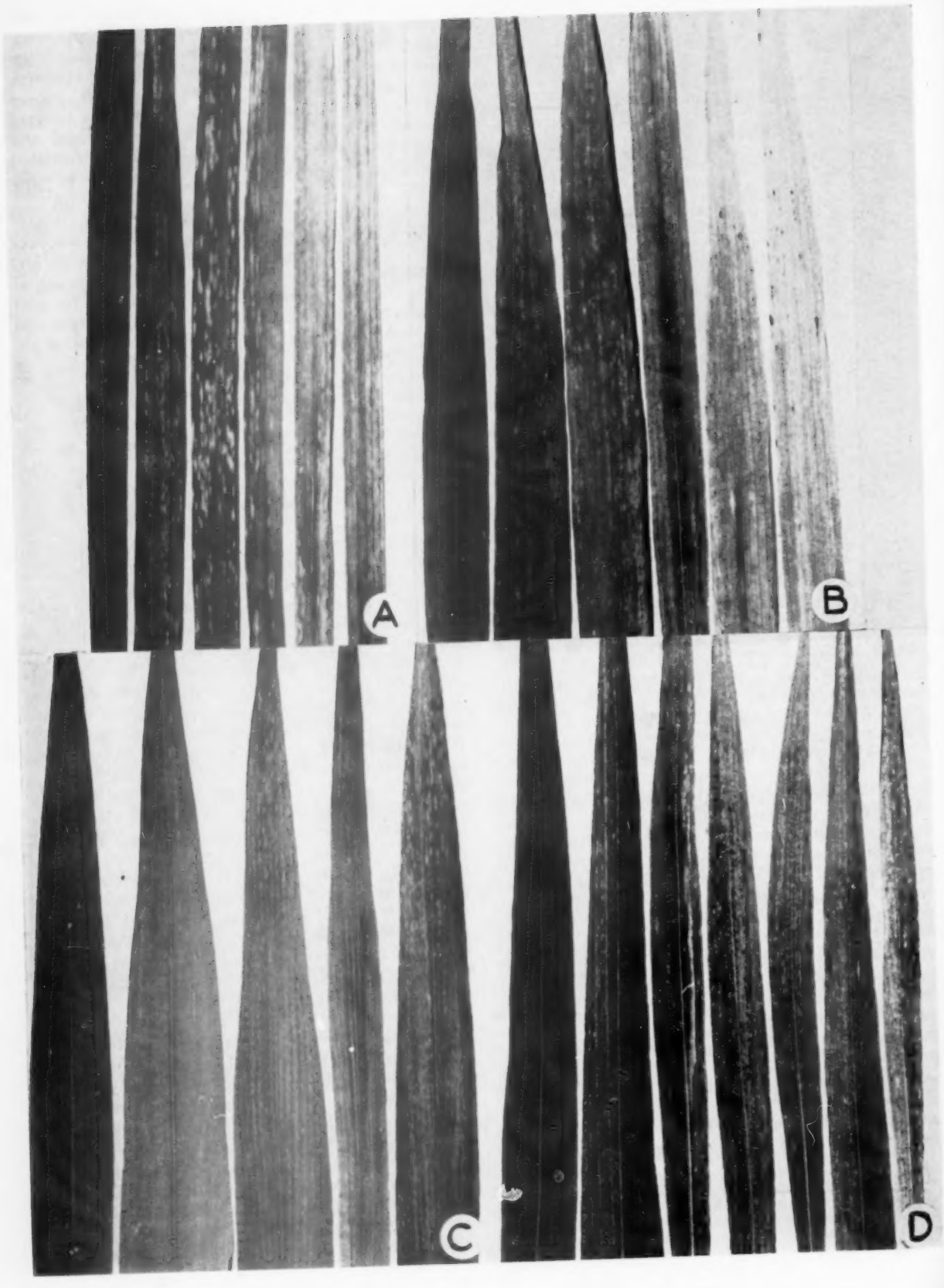
FIG. 2. A cage to protect colonies of *Aceria tulipae* on wheat from invasion by predaceous mites, *Typhlodromus cucumeris* Oudms.

were constructed of clear cellulose nitrate plastic sheets. The ends of the cylinders were reinforced with metal rings cemented to the plastic. White nylon taffeta cloth was cemented over the top and over 2 ventilator holes cut in the sides of the cylinders. Ordinarily these cages were used by placing them over plants growing in soil in 6-in. pots, the open base of the cage being pressed into the soil in which the plants were growing. These cages satisfactorily retained the eriophyid mites and excluded aphids and even the powdery mildew fungus; however, they did not exclude an active predaceous mite, *Typhlodromus cucumeris* Oudms.,[4] which became a persistent and destructive invader of the cultures of *A. tulipae*. Special cages were employed therefore to exclude this predator (Fig. 2). Instead of pots, ½-gal. Mason jars were employed as soil containers. The metal base of the cylindrical cages was constructed in such a manner that it could be soldered securely to the metal screw ring of the Mason jar. With the rubber gasket in place on the rim of the glass jar, the cage could be screwed snugly on the jar, leaving no openings through which even tiny pests could enter. Watering of the soil in the glass jar without removing the cage was facilitated by bending a ¼-in. copper tube into an "S" shape so that it could be inserted through a hole in the metal base of the cage and soldered securely. In these cages, cultures remained free from the predators much longer than in other cages.

SYMPTOMS CAUSED BY ISOLATES OF SPOT MOSAIC VIRUS.—Symptoms of spot mosaic appeared 3–4 days after infective mites were placed on Kharkov 22 MC wheat in the 1- to 3-leaf stage in a greenhouse at about 70°F. The earliest symptoms produced by most isolates consisted of light green spots about 0.5 mm × 1 mm in size on the youngest leaf of the plant. The faint chlorosis intensified to distinct yellow or white, and the spots became necrotic. They usually enlarged and coalesced to form chlorotic areas, and often the entire leaf became first chlorotic and then necrotic (Fig. 3, A). Some isolates produced a vaguely defined mottle instead of distinct spots; occasionally, fine streaks were apparent. The more virulent isolates caused necrosis of leaf tissue, stunting, and even death of plants (Fig. 1). The most virulent isolate was so pathogenic to wheat that it was difficult to maintain in culture and was lost within a year of its isolation. Other isolates caused spotting of varying degrees of intensity but had a relatively mild effect on the plant. Such isolates have been easily maintained in culture for the 3 years since they were isolated.

ACQUISITION OF SPOT MOSAIC VIRUS BY NONINFECTIVE MITES.—Difficulty was experienced in determining whether noninfective mites could become infective with spot mosaic virus by feeding on diseased plants. It was not easy to obtain diseased wheat plants entirely free from mites. Attempts were made to eliminate mites by repeated fumigation with a tetraethyldithiopyrophosphate fumigant. Plants protected from reinfection by mite-proof cages and fumigated at 5- to 7-day intervals for 4–5 weeks were not necessarily free from mites. If such plants were held for several weeks without fumigation, mites could sometimes be found on them. The most successful method was to fumigate the plants at frequent intervals to reduce surviving mites to as few as possible; then when the plants had produced substantial crowns, the aboveground parts were cut off just above the crowns and discarded. The pots of soil containing the roots and crowns were again fumigated, caged, and set in a cool greenhouse to encourage the growth of new shoots. Unfortunately, plants infected with the more virulent strains of spot mosaic virus did not survive this drastic treatment, but some of the plants infected with moderate or mild strains produced new leaves with spot mosaic symptoms. Repeated checking for several weeks before noninfective mites were applied and the continued checking of control plants that were not reinfested indicated that all mites were eliminated from the plants. Plants that were infested

[4] Specimens were identified by Dr. H. H. J. Nesbitt, Chairman, Department of Biology, Carleton College, Ottawa, Ontario, Canada.


A
B
C
D

with noninfective mites were freed from their infestation in the same manner and used as controls in the subsequent experiment on the acquisition of the virus.

The test for the acquisition of the virus by noninfective mites was made with infected and noninfected plants that were freed from mites. Noninfective mites were applied to these plants, and 7 days later several mites were removed and placed on young test plants. In 3 experiments, spot mosaic symptoms developed on more than ⅔ of the 15 to 20 plants to which mites from plants infected with isolates of spot mosaic were transferred. No symptoms developed on any of 15 to 20 control plants on which mites from the noninfected controls had fed.

TRANSMISSION OF SPOT MOSAIC VIRUS BY VARIOUS STAGES OF A. TULIPAE.—Tests were made of the infectiveness of *A. tulipae* at different stages of development. The mites were reared on wheat plants infected with each of 6 different isolates of spot mosaic virus. For each stage of the mite (except the egg), 2 mites were transferred to a single wheat seedling growing in a large test tube. In tests with eggs, 10 were placed on each seedling. Five plants were infested in this way for each stage of the mite being tested for the transmission of each of the 6 isolates of spot mosaic virus. Mites from a noninfective colony were similarly put on test plants to serve as controls. All plants were fumigated 2 weeks later. The results (Table 1) indicated that mites at all stages of development, from shortly after hatching until they are adults, may be infective. Infectivity is retained during molting, but not through the egg stage. Regardless of their origin, eggs that hatch on healthy plants produce noninfective mites.

HOSTS OF SPOT MOSAIC.—The results of tests of the susceptibility of a number of annual and perennial grasses to infection with wheat streak mosaic virus transmitted by *A. tulipae*, as well as by manual inoculation, were reported earlier.[3] Susceptibility to spot mosaic was tested with a similar selection of grasses. The grasses were grown from seed planted in steam-sterilized soil in 6-in. pots. The plants were infested in the 2- to 3-leaf stage. It was necessary to keep them well protected from accidental infection by other viruses and to maintain well-protected controls with noninfective mites. Instead of dispersing the mites with fans in the greenhouse as was done in some of the tests with wheat streak mosaic virus, the plants were infested by placing several mites, or pieces of leaves bearing mites, on each of them. The plants were then caged. The annual grasses were fumigated with a tetraethyldithiopyrophosphate fumigant 1 week after infestation, then removed from the cages and placed on greenhouse benches. Weekly fumigations were necessary to ensure against multiplication and spread of mites. The perennial grasses were kept in cages and not fumigated. Notes on symptoms were taken 2–3 weeks after infestation with mites. To check for the presence of a mechanically transmissible virus like that of wheat streak mosaic, all hosts were then tested by mechanical inoculation to Kharkov wheat.

TABLE 1.—*Transmission of spot mosaic virus to wheat seedlings by various stages of Aceria tulipae that had been reared on infected plants*

Isolate number	Number out of 5 test plants infected after being fed upon by indicated stage[a]					
	Egg[b]	First nymph	First molt	Second nymph	Second molt	Adult
1	0	3	4	5	5	5
5	0	4				4
10	0	4				3
13	0	3	1	5	2	4
50	0	3				5
116	0	5	4	5	3	5
Noninfective	0	0	0	0	0	0

[a] Except for the egg stage, 2 mites were transferred to each test plant and allowed to feed for 2 weeks.

[b] Ten eggs from mites reared on infected plants were placed on each test plant and the newly hatched mites allowed to feed for 2 weeks.

The results of tests with spot mosaic virus on a number of cultivated and wild grasses, all of which can be infected with wheat streak mosaic virus by the mite *Aceria tulipae*, are shown in Table 2. The ability of the mites to survive and increase on each grass also is indicated. Particular care was necessary to evaluate symptoms of spot mosaic because of the chlorotic symptoms produced by noninfective as well as infective mites on some of the hosts. The barley varieties exhibited a chlorotic mottle in response to the feeding of all mites; however, this mottle did not occur on new leaves produced after most of the mites had been killed by fumigation. Conversely, a chlorotic spotting did occur on new leaves produced after fumigation on barley plants that had been infested with mites that carried spot mosaic virus (Fig. 3, B). This indicated that the barley was mildly susceptible to the spot mosaic virus, and that in addition, the feeding of the mites directly caused chlorotic spots.

Chlorosis can become quite pronounced on wheat when noninfective mites multiply profusely. In the present tests, the mites were allowed to feed and reproduce on the plants for only 1 week before they were killed. Under these circumstances, only slight

FIG. 3. Wheat spot mosaic symptoms on leaves of A) Kharkov 22 MC winter wheat, B) Trebi barley, C) Northern Cross corn, and D) Hungarian millet. In each case the leaf on the extreme left is healthy.

TABLE 2.—*Reaction of annual grasses to Aceria tulipae and to spot mosaic virus*

Host species	Variety	Maintenance of mites for 1 week[a]	Chlorotic spotting[b] induced by		Reaction to spot mosaic virus[b]
			Noninfective mites	Infective mites	
CULTIVATED GRASSES:					
Avena sativa L.	New Garry	+	tr	tr	0
Avena sativa L.	Victory	+	tr	tr	0
Echinochloa crusgalli (L.) Beauv.	Japanese Millet	+	tr	sl	0
Hordeum vulgare L.	Atlas	++	0	mod	sl
Hordeum vulgare L.	Trebi	++	sl	mod	sl
Hordeum vulgare L.	Vantage	++	sl	sev	sl
Panicum miliaceum L.	Proso Millet	0	tr	tr	0
Secale cereale L.	Dakold	++	0	sl	sl
Setaria italica (L.) Beauv.	Hungarian Millet	+	tr	mod	mod
Triticum dicoccum Schrank	Vernal	++	0	mod	mod
Triticum durum Desf.	Mindum	+++	tr	sev	mod
Triticum timopheevi Zhukov		++	0	mod	mod
Triticum aestivum L. (spring type)	Rescue	+++	sl	sev	sev
Triticum aestivum L. (spring type)	Thatcher	+++	tr	sev	sev
Triticum aestivum L. (winter type)	Jones Fife	+++	tr	sev	sev
Triticum aestivum L. (winter type)	Kharkov	+++	0	sev	sev
Zea mays L.	Golden Rush	+	tr	mod	mod
Zea mays L.	Northern Cross	+	tr	mod	mod
WILD GRASSES:					
Avena fatua L.	Wild Oats	+	tr	tr	0
Bromus japonicus Thunb.	Japanese Chess	0	0	0	0
Bromus secalinus L.	Cheat	0	0	0	0
Bromus tectocum L.	Downy Brome	0	0	0	0
Digitaria sanguinalis (L.) Scop.	Crab Grass	0	0	0	0
Echinochloa crusgalli (L.) Beauv.	Barnyard Grass	+	0	0	0
Eragrostis cilianensis (All.) Lutati	Stink Grass	+	tr	mod	mod
Panicum capillare L.	Witch Grass	0	0	0	0
Setaria verticillata (L.) Beauv.	Bristly Foxtail	+	0	mod	mod
Setaria viridis (L.) Beauv.	Green Foxtail	+	0	mod	mod

[a] + = few mites; ++ = moderate survival of mites; +++ = abundance of mites; 0 = no mites.
[b] tr = trace of spotting; sl = slight spotting; mod = moderate spotting; sev = severe spotting; 0 = no spotting.

chlorotic symptoms or no symptoms resulted from the feeding of the noninfective mites. In contrast, infective mites caused distinct chlorotic and sometimes necrotic spots on the wheat varieties tested. The symptoms were most severe on *Triticum aestivum*, and successively less severe on *T. durum*, *T. dicoccum*, and *T. timopheevi*. *Zea mays*, *Setaria italica*, *S. viridis*, and *S. verticillata* resembled the wheat varieties in that little or no chlorosis was induced by noninfective mites, but a marked chlorotic spotting was induced by the infective mites (Fig. 3, C, D).

Several perennial grasses did not support mites for as long as 1 week and did not develop chlorotic symptoms. These were *Agropyron elongatum* (Host.) Beauv., *A. repens* (L.) Beauv., *A. smithii* Rydb., *A. trichophorum* (Link) Richt., *A. trachycaulum* (Link) Malte, *Bromus inermis* Leyss., *Elymus canadensis* L., *Poa pratensis* L., and *Stipa comata* Trin. & Rupr. Plants of *Agrostis alba* L. that were infested with either infective or noninfective mites developed a mild spotting even though no mites were found 1 week after they were infested. A few mites survived for more than a week on *Agropyron cristatum* (L.) Gaertn., *A. intermedium* (Host) Beauv., *Festuca rubra* L., and *Poa compressa* L.; however, neither infective nor noninfective mites produced more than a very mild chlorotic spotting on any of the plants. The only perennial grasses in these tests on which *Aceria tulipae* survived for more than 3 weeks and increased in number were *Festuca elatior* L. and *Phleum pratense* L., but these plants showed no evidence of symptoms attributable to spot mosaic virus.

INFECTION OF TRITICUM × AGROPYRON HYBRIDS WITH SPOT MOSAIC VIRUS. — Forty-one Triticum × Agropyron hybrids were tested for reaction to virus transmitted by mites originating from naturally diseased wheat. Some of these were susceptible to wheat streak mosaic virus, some were symptomless carriers, and others developed only a chlorotic speckling on the leaves. Some of the speckling was a local reaction that also could be produced by mechanical inoculation with wheat streak mosaic virus; however, some of the hybrids on which infective mites had fed continued to develop chlorotic spots on new leaves produced even after the mites had been eliminated. Noninfective mites were placed on such plants, and 1 week later mites were removed and placed on Kharkov wheat seedlings. Spot mosaic symptoms developed on the seedlings, indicating that spot mosaic virus was the cause of spotting on several hybrids tested.

DISCUSSION.—Spot mosaic virus, like streak mosaic virus, is transmitted in nature by *Aceria tulipae*, and the 2 viruses usually occur together in the field. The

fact that streak mosaic virus is mechanically transmissible whereas spot mosaic is not has made it possible to distinguish them. Once separated, several differences between the two are readily demonstrated. At greenhouse temperatures around 70°F, the first symptoms of spot mosaic become evident in 3 or 4 days. Those of streak mosaic do not appear until 6–8 days after inoculation. The first symptoms of spot mosaic are distinct, separated chlorotic spots. Those of streak mosaic are chlorotic dashes and streaks parallel to the leaf axis. Although the 2 viruses have a number of grass hosts in common, the host ranges are not identical. Some of the Triticum × Agropyron hybrids that appear to be immune to streak mosaic virus are susceptible to spot mosaic virus.

The detection of spot mosaic virus in association with streak mosaic virus, a combination causing a disease that was previously attributed solely to the latter, probably will remain of academic rather than practical significance in southern Alberta as long as the disease can be controlled so well by cultural methods.[3] If host resistance becomes a factor in mosaic control, spot mosaic virus may become a complicating factor because of the differences in the host reactions to the 2 viruses.

PLANT PATHOLOGY SECTION
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A CORM ROT OF GLADIOLUS CAUSED BY RHIZOPUS ARRHIZUS

W. D. McClellan[2]

SUMMARY

A light brown colored soft rot of gladiolus corms caused by *Rhizopus arrhizus* A. Fisch. may occur when freshly harvested corms are heat-cured at 85° or 95° F; the greatest amount of rot occurs at 95° F. Losses are greatest when corms are stored under humid conditions and when corms are injured. The 5 varieties of gladiolus tested were susceptible in the following order: Picardy; October Sunshine; Spotlight; Chamouny; and Spic and Span.

During an investigation of curing practices in relation to the development of corm rot and yellows caused by *Fusarium oxysporum* f. *gladioli* Snyd. & Hans. in freshly harvested gladiolus corms, a light brown soft rot caused by a species of *Rhizopus* was encountered. This rot, briefly discussed by Bald[3] and by the author[4], has not been described previously. To gain more information on this rot, tests were made in 1954 and 1955.

SYMPTOMS.—The only discoloration of gladiolus corm tissue rotted by Rhizopus is a light brown coloring of the flesh (Fig. 1). Except for this slight darkening, rotted corms look normal; the most effective way to detect Rhizopus rot is to squeeze the corm gently. Occasionally under high-moisture conditions, typical Rhizopus spores and hyphae appear at the base, at the stem end, or in one of the cuts on the sides of the corms. Little or no odor accompanies the rot unless soft-rot bacteria have gained entrance; then a putrid odor is evident. Most of the corms of the variety October Sunshine, which remained in the curing room longer than the other varieties, had become very hard, dry mummies. Rhizopus was fruiting abundantly under the husks when they were examined. Rotted corms of the other varieties became mummified when left in a warm, dry room.

MATERIALS AND METHODS.—Five varieties of gladiolus, Picardy, October Sunshine, Chamouny, Spotlight, and Spic and Span, were used in the tests in 1954, and one variety, Spotlight, was used in 1955. The corms were field-grown at Beltsville from corms and cormels and only No. 1 or larger corms were used. Immediately after harvest, the corms of each variety were sorted into lots of uniform size. There were 5 lots, or replicates, for each treatment and for each variety. During the 2 years, 410 lots were used. After sorting, the corms in some of the lots were injured by cutting out small wedge-shaped segments from 3 sides. Some of the lots of injured corms and some of the uninjured ones were then dipped in a spore and mycelial suspension of Rhizopus previously isolated from rotting gladiolus corms. The lots of corms were further divided and placed under different curing conditions.

TESTS IN 1954.—In 1954 the curing conditions were 1) 95°F with a high relative humidity (approximately 95 per cent); 2) 95° with a low relative humidity

[1] Accepted for publication July 30, 1956.

[2] Pathologist, Horticultural Crops Research Branch, Agricultural Research Service, U. S. Department of Agriculture, Beltsville, Maryland.

[3] Bald, J. G. 1953. Gladiolus diseases. A progress report on current research compiled from discussions at the Eighth Annual Convention, North American Gladiolus Council, Cleveland, Ohio. January 15-18. (Mimeographed).

[4] McClellan, W. D. 1956. A Rhizopus rot of gladiolus corms. (Abs.) Phytopathology 46: 468.

(20–30 per cent); and 3) 65° with a high humidity (95 per cent). Picardy and Chamouny corms were harvested October 26; Spic and Span and Spotlight, November 5; and October Sunshine, November 9. October Sunshine corms were cured for 20 days and all others for 13 days. There were 5 corms of each variety per replicate. The rotted corms were counted, at the end of the curing period, and all nonrotted corms of Picardy, October Sunshine, and Chamouny were then placed in the 95° and 95 per cent relative humidity chamber until December 14 to determine whether further rot would develop. Rotted corms from these lots were kept in a warm, dry room for further observation.

Tests in 1955.—Spotlight corms of 2 sizes were used in 1955; the so-called "new" corms were No. 1 or larger corms from cormels planted in 1955. These corms were harvested and sorted into lots of 22 for each replicate and treatment. The new corms were harvested October 28, sorted, inoculated, and placed under the different curing conditions for 16 days. The jumbo corms were dug and treated November 17 and cured for 11 days. The curing conditions were 95° and 85°F with both high and low humidity at each temperature and 75° and 65° with high humidity. High humidity was obtained by wrapping in polyethylene bags, which maintained a nearly saturated atmosphere. Low-humidity treatments consisted of curing unwrapped corms in each temperature chamber.

Half the corms at 95° and 85° and all those at 75° and 65° were injured before curing; all were inoculated with Rhizopus. An eleventh lot was injured, cured at 85° with high humidity for 5 days, inoculated with Rhizopus, and given a second curing at 95° with high humidity.

Results.—In the 1954 tests the percentages of corms rotted by Rhizopus were as follows: Picardy, 21.3; October Sunshine, 20.6; Spotlight, 14.0; Chamouny, 9.3; and Spic and Span, 5.3.

The 1954 results with Picardy and Spic and Span are summarized in Table 1. Similar results were ob-

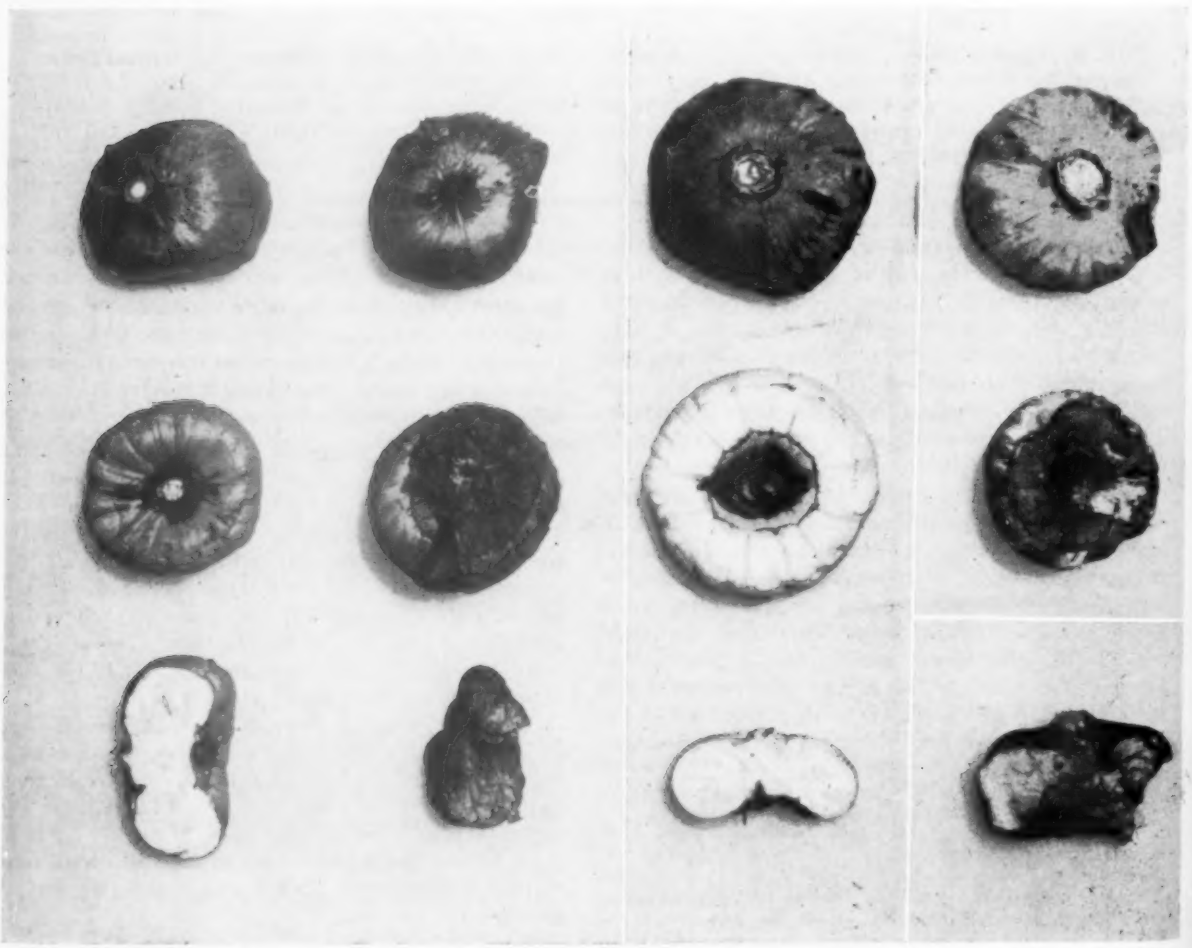

Fig. 1. Healthy and diseased gladiolus corms: Pairs at left, Picardy; pairs at right, Spic and Span. In each group the corm at right is infected by Rhizopus and the other is healthy. The top row shows the tops of unhusked corms, the middle one the bottoms of husked corms, and the bottom row vertically split husked corms.

TABLE 1.—*Rhizopus rot of corms of 2 varieties of gladiolus after a 13-day curing in 1954*[a]

Curing conditions			Picardy	Spic and
Temperature	Relative humidity	Corms inoculated	corms rotted[b]	Span corms rotted
°F	per cent		per cent	per cent
95	95	Yes	28	12
95	95	No	10	0
95	20-30	Yes	26	4
95	20-30	No	0	0
65	95	Yes	0	0
65	95	No	0	0

[a] The injured and uninjured corms are combined in the percentages given here.
[b] LSD at odds of 19:1, 17.7.

tained with the other 3 varieties. Ten per cent of the noninoculated Picardy corms cured at 95° and 95 per cent relative humidity rotted, indicating that some natural infection had occurred. There was more rot during curing at a high humidity than at a low humidity, and a temperature of 65° was apparently too low to permit infection. After the curing periods (20 days for October Sunshine), apparently healthy corms of 3 varieties from the different treatments were grouped by variety and placed in the room kept at 95°F and 95 per cent relative humidity—Picardy and Chamouny for 36 additional days and October Sunshine for 16 days. Rotting occurred in 18.6 per cent of the Picardy corms, in 7.4 per cent of the October Sunshine, and in 43.5 per cent of the Chamouny, indicating that the previous treatments were not effective in preventing further rot.

The 1955 results with Spotlight are summarized in Table 2. A temperature of 95°F with a high relative humidity was the most favorable condition tested for promoting rot. Corms maintained at a low humidity were much less susceptible; in fact, there was no rot at 85° with low humidity. No rot occurred at 65° or 75°. Injured corms were much more susceptible than uninjured ones, but lack of visible injury did not prevent infection. Curing at 85° for 5 days prior to inoculation did not prevent infection.

A typical Rhizopus was isolated consistently from the rotting corms, and this fungus reproduced the disease. Growth on potato-dextrose agar was very rapid at high temperatures, covering a 90-mm petri dish in 24 hours at 95°F. Little or no growth occurred at 45°; in 72 hours, half a 90-mm petri dish was covered at 55° whereas the entire dish was covered at 65°, 75°, or 85° in 72 hours. Numerous spores, 5.5–7.5μ in diameter, were produced at 75°, 85°, and 95°, but none were produced at 55° or 65°. A transfer of this Rhizopus was sent to C. W. Hesseltine, Culture Collection Unit, Fermentation Section, Northern

TABLE 2.—*Rhizopus rot in inoculated Spotlight gladiolus corms after curing in 1955*

Curing conditions			New	Jumbo
Temperature	Relative humidity	Corms injured	corms rotted[a]	corms rotted[b]
°F			per cent	per cent
95	High	Yes	100.0	88.4
95	High	No	80.8	55.0
95	Low	Yes	7.4	8.4
95	Low	No	2.0	5.0
85	High	Yes	49.2	58.4
85	High	No	8.4	31.6
85	Low	Yes	0.0	0.0
85	Low	No	0.0	0.0
75	High	Yes	0.0	0.0
65	High	Yes	0.0	0.0
95[c]	High	Yes	30.6	16.8

[a] Largely No. 1 corms produced from cormels planted in 1955. LSD at odds of 19:1, 15.4.
[b] Corms produced from No. 3 and 4 corms planted in 1955. LSD at odds of 19:1, 23.8.
[c] Corms cured at 85° for 5 days prior to inoculation and curing at 95°.

Utilization Research Branch, Peoria, Illinois, who identified it as *Rhizopus arrhizus* A. Fisch. This is Dr. Hesseltine's lyophilized culture A-6614.

DISCUSSION.—High temperature curing of gladiolus corms may result in losses from Rhizopus rot, but these losses probably will not be extensive. Few commercial gladiolus growers cure their gladiolus at 95°F, and if they do, their purpose is to dry the adhering soil by passing moving warm air over the corms for 24–48 hours. Gladiolus corms usually are cured at 85° with a humidity approaching 70 per cent, but Bald[5] stated that his best treatment for developing periderm and cuticle resistant to infection was to cure the corms immediately after digging at 95° and at a relative humidity of 80 per cent.

In many respects, Rhizopus rot of gladiolus corms is similar to the Rhizopus rot of sweet potato roots described by Harter and Lauritzen[6]. Sweet potato roots also behave much like gladiolus corms insofar as suberization and wound periderm formation are concerned[7]. It is concluded that although gladiolus corms can be rotted by *R. arrhizus* at high temperatures and high humidities, this rot presumably will be of minor importance in the commercial culture of gladiolus.

[5] Bald, J. G. 1953. Control of disease by heat-curing and dipping gladiolus corms. Phytopathology 43: 141-155.
[6] Harter, L. A., and Lauritzen, J. L. 1921. The decay of sweet potatoes (Ipomoea batatas) produced by different species of Rhizopus. Phytopathology 11: 279-284.
[7] Artschwager, E., and Ruth Colvin Starrett. 1931. Suberization and wound-periderm formation in sweet potato and gladiolus as affected by temperature and relative humidity. Jour. Agr. Res. 43: 353-364.

EFFECTS OF FREQUENCY OF SULFUR DIOXIDE FUMIGATION DURING STORAGE ON DECAY AND FUMIGATION INJURY IN EMPEROR GRAPES[1]

John M. Harvey

SUMMARY

Normally, California table grapes are fumigated initially with a 1 per cent concentration of sulfur dioxide gas for 20 minutes and subsequently at intervals of 7 to 10 days during storage with a ¼ per cent concentration of gas. Fumigation greatly reduces decay, but may also cause chemical injury to the fruit. A series of experiments was conducted during a 3-year period to determine the amount of decay and injury that occurred when grapes were fumigated during storage at intervals of 7, 14, or 21 days. The effect of frequency of fumigation as related to harvest date and length of storage also was determined.

A consistent relation between frequency of fumigation during storage and sulfur dioxide injury could not be demonstrated in these studies. There was some evidence that mechanical injuries, which allowed the gas to penetrate the berries more readily, and high initial concentration of gas were more important as causes of fumigation injury than was frequency of fumigation during storage. When grapes were stored for long periods, more decay developed in those that received the less frequent fumigations than in those that were fumigated more often. The frequencies of fumigation used in this study had no significant effect on decay in grapes stored for short periods.

The results also indicated that more decay occurred in grapes harvested late in the season than in those picked early. There was also a positive correlation between length of storage and percentage decay.

Losses from decay, caused chiefly by the gray mold fungus, *Botrytis cinerea* Pers., may be considerable in California table grapes held in cold storage for long periods. Other fungi that cause decay in storage are *Cladosporium herbarum* Link ex Fr. and species of *Alternaria* and *Stemphylium* (4, 5, 7, 21). Normally, California table grapes are fumigated with 1.0 per cent sulfur dioxide gas for 20 minutes when they are placed in storage; during storage they are fumigated at 7- or 10-day intervals with a ¼ per cent concentration of gas for 20 minutes. Although these fumigations reduce the development of decay in stored grapes, they may also injure the fruit (1, 2, 12, 14, 15, 16, 22, 25). Injury may be the result of using too high a concentration of gas, of exposing the fruit too long to the gas, or of fumigating too often during storage. The purpose of this study was to determine the effect of frequency of fumigation during storage on decay and on sulfur dioxide injury and to relate these factors to harvest date and length of storage.

METHODS.—Grapes of the Emperor variety were used for these studies, since Emperor is the principal storage variety grown in California. The experiments were begun in 1951 and were repeated in 1952 and 1953.

Previous studies conducted with table grapes had indicated that it is difficult to obtain uniform samples for experimental use. Great variability in the percentage of decay that developed in storage occurred among individual grape clusters. In 1951, the samples were prepared by carefully compositing them from individual clusters taken from a large number of separate field boxes. Eight replicates were used to allow for the variability in clusters; however, even with these precautions, a rather large LSD (5.5 per cent at the 5 per cent level) was obtained. Greater uniformity was obtained in 1952 and 1953 when clusters were cut into smaller units; from these divided clusters, 8 replicated samples of approximately 7 lb. each were composited for each treatment, each examination, and each harvest. The identity of each sample was maintained by enclosing it in a single thickness of cheesecloth. One sample for a 3-month examination and another for a 5-month examination were placed in each standard 28-lb. lug box. The remainder of the space in each box was filled with extra grapes to maintain normal moisture conditions. When the fruit was removed for the 3-month examination, it was replaced with additional extra fruit to refill the lug box. The 8 replicated boxes for each treatment and each harvest were randomly positioned in storage. During storage, the temperature was maintained at 31–32°F and the relative humidity was held at 85–95 per cent.

Since the amounts of decay that develop in stored grapes normally vary with the date of harvest, both an early and a late harvest of grapes were made. This provided a test of the various treatments under conditions of both low and high decay incidence and also allowed a determination of the effect of the gas on grapes of different maturities. To insure that decay organisms were uniformly present on the grapes, the fruit was inoculated with spores of *Botrytis cinerea*. Inoculation was made by dusting the grapes with spores collected from pure cultures of *B. cinerea* grown on petri dishes. An apparatus similar to the one described by Tervet et al (24) was used for collecting and dispersing the spores over the fruit.

All the grapes used in these experiments were fumi-

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Acknowledgment is made of the assistance of A. Lloyd Ryall, W. R. Barger, M. Uota, and Paul Rood in the preparation of test samples and in their detailed examination after treatment.

gated initially with a 1 per cent concentration of sulfur dioxide gas for 20 minutes. During storage, the various lots of grapes were fumigated with a ¼ per cent concentration of gas for 20 minutes at intervals of 7, 14, or 21 days. In 1952 an additional treatment was included; in this the grapes received a second 1 per cent fumigation 2 days after the initial one and during storage received the regular ¼ per cent fumigation at intervals of 14 days. Occasionally, some commercial lots receive 2 fumigations with 1 per cent gas as a result of being treated in the field boxes immediately after they are brought to the packing house and again after packing. Some lots are refumigated also with a 1 per cent concentration of gas in the refrigerator cars before shipment. Since grapes cannot be stored for long periods (1) without sulfur dioxide fumigation, an unfumigated check lot was not included.

Immediately after the fruit was removed from storage at the 3- and 5-month periods, the samples were weighed and decay examinations made. All decayed berries were removed from the clusters, and those from each sample were weighed and the percentage decay calculated. After the remaining fruit from each sample had been held for 1 day at room temperature, it was examined for fumigation injury. The latter is more apparent after the fruit is exposed to room temperature than immediately after its removal from cold storage. Sulfur dioxide injury is primarily manifested in berries of the Emperor variety as sunken, bleached areas near the capstem attachment (14, 21).

RESULTS.—*Decay.*—The effects on decay of frequency of fumigation (treatment) during storage, length of storage, and time of harvest are summarized in Table 1. The first-order interactions (3, 23) of the main effects also are shown. Differences described in this paper as "significant" are statistically significant.

1) *Experiments in 1951.*—As normally would be expected, more decay occurred in late-harvested than in early-harvested fruit and more in fruit stored 5 months than in that stored 3 months. An analysis of variance of the data showed that these differences in decay were significant. The interaction between harvest date and length of storage was significant at the 5 per cent level. Thus, the difference in the percentage of decay that developed in fruit stored 3 or 5 months was significant in late-harvested but not in early-harvested fruit. Significantly more decay developed in late-harvested grapes stored only 3 months than in early-harvested grapes stored 5 months. No significant differences in amount of decay were found in the replicates, although they exhibited considerable variability. Frequency of fumigation during

TABLE 1.—*Decay of Emperor grapes harvested on different dates and fumigated at various intervals during different periods of storage*

Year	Harvest	Decay[a] in grapes fumigated at indicated intervals during 3 or 5 months' storage							Average decay in grapes stored for indicated period			Average decay in grapes from each harvest	
		7-Day interval		14-Day interval		21-Day interval							
		3-Month storage	5-Month storage	3-Month storage	5-Month storage	3-Month storage	5-Month storage	LSD (5%)	3-Month storage	5-Month storage	LSD (5%)	Amount	LSD (5%)
		%	%	%	%	%	%	%	%	%	%	%	%
1951	Early (Oct. 18)	4.2	2.6	4.8	4.9	4.1	4.7	5.5[b]	4.3	4.1	2.8[c]	4.2	2.0[d]
	Late (Nov. 15)	7.5	13.6	9.2	16.3	9.9	10.2		8.9	13.4		11.1	
	Average	5.9	8.1	7.0	10.6	7.0	7.5	N.S.	6.6	8.8	2.0[d]		
	Treatment average	7.0		8.9		7.3		N.S.					
1952	Early (Oct. 14)	0.5	1.1	0.4	1.8	0.3	2.3	0.7[b]	0.4	1.7	0.4[c]	1.1	0.3[d]
	Late (Nov. 3)	0.6	3.2	0.6	3.6	0.6	4.1		0.6	3.7		2.1	
	Average	0.6	2.2	0.5	2.7	0.5	3.2	0.5[e]	0.5	2.7	0.3[d]		
	Treatment average	1.3		1.6		1.9		N.S.					
1953	Early (Oct. 13)	1.9	2.6	1.6	3.0	1.1	3.0	2.5[b]	1.5	2.9	N.S.	2.2	1.0[d]
	Late (Nov. 2)	2.0	2.6	1.4	3.5	2.6	7.7		2.0	4.6		3.3	
	Average	2.0	2.6	1.5	3.3	1.9	5.4	1.7[e]	1.8	3.7	1.0[d]		
	Treatment average	2.3		2.4		3.6		N.S.					

[a] Except those labeled as averages, all figures are means of 8 replicated samples.
[b] LSD for comparison of any 2 of the 12 figures (in this line and in that immediately below) to the left.
[c] LSD for comparison of any 2 of the 4 figures (in this line and in that immediately below) to the left.
[d] LSD for comparison of the 2 figures (in this line and/or in that immediately below) to the left.
[e] LSD for comparison of any 2 of the 6 figures to the left.

storage had no significant effect on decay in the 1951 test.

2) *Experiments in 1952.*—The date of harvest and the length of storage had significant effects on decay as in the previous year (Table 1). After 3 months' storage there was very little difference in decay in early-harvested and late-harvested grapes, but after 5 months significantly more decay was present in late-harvested than in early-harvested fruit. Although the main effect of frequency of fumigation (treatment) was not significant, the interaction between treatment and length of storage was significant at the 5 per cent level. Thus, after 3 months' storage, treatment had little effect on decay, but after 5 months the differences between treatments were significant. After the longer storage period, progressively more decay was found as the interval between fumigations was extended. Fruit fumigated at intervals of 21 days had significantly more decay than fruit fumigated at 7-day intervals, irrespective of harvest date. The difference in decay in fruit fumigated at 14-day intervals and that fumigated at 7-day intervals, however, was not significant in late-harvested fruit and was barely significant in early-harvested fruit. Differences in decay in the replicated samples were not significant.

The 1 additional 1952 treatment in which the grapes received 2 initial 1 per cent fumigations did not result in a significant reduction in decay. Grapes that received this treatment averaged 1.4 per cent decay when removed from storage.

3) *Experiments in 1953.*—Differences in decay as affected by length of storage were again significant, but differences due to harvest date were not. As in the previous years, the main effect of treatment was not significant; however, the interaction between treatment and length of storage was significant at the 5 per cent level. Thus, in fruit stored for 5 months, significantly more decay occurred when the fumigation interval was 21 days than when it was 14 or 7 days and slightly more decay developed in grapes fumigated at 14-day intervals than at 7-day intervals, but this difference was not great enough to be significant.

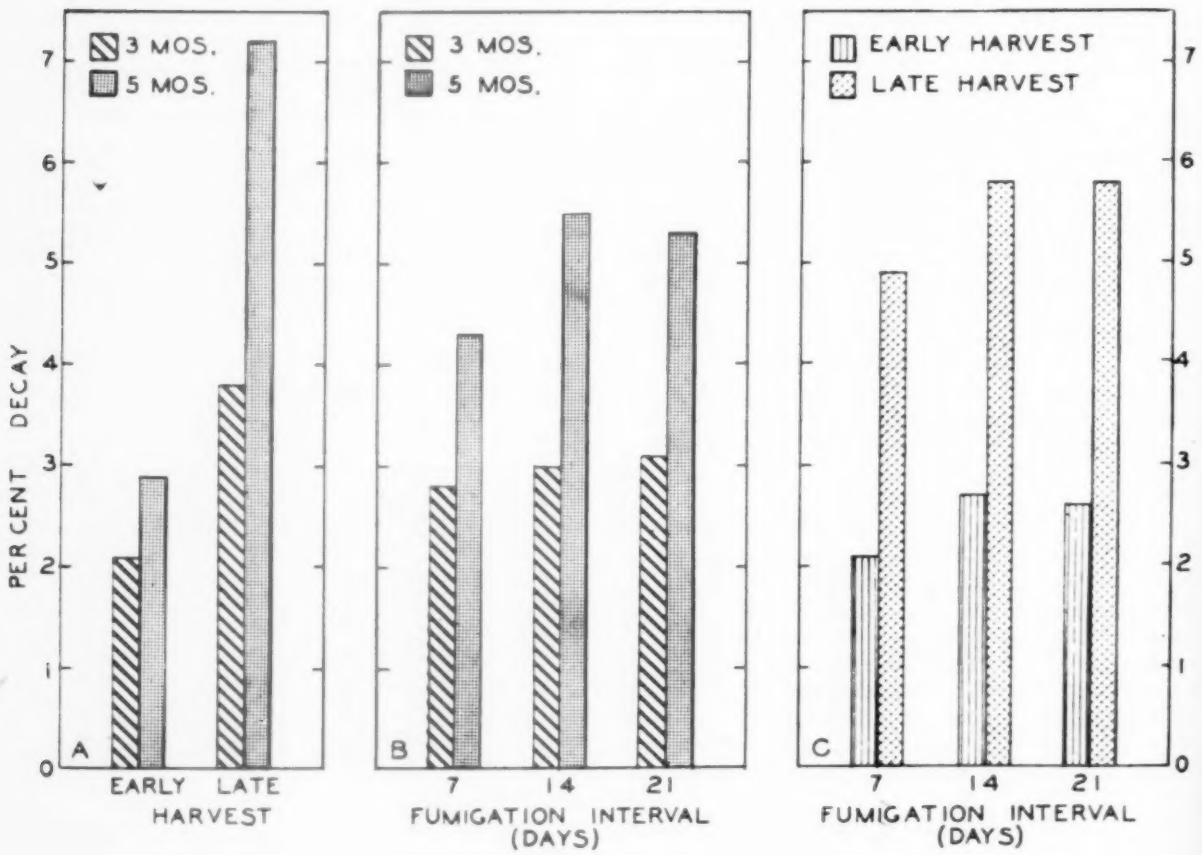

FIG. 1. Three-year average (1951-53) of decay in Emperor grapes; A) relation of harvest date and length of storage; B) relation of fumigation interval and length of storage; C) relation of fumigation interval and harvest date.

Frequency of fumigation had no significant effect on decay in grapes stored for only 3 months or on fruit harvested early in the season. Differences in decay in the replicated samples were not significant.

4) *Combined data for 3 years.*—Three-year averages of the decay in grapes stored for 3 or 5 months and fumigated at intervals of 7, 14, or 21 days during storage are shown in Figure 1, B. An analysis of variance of the combined data for the 3-year period indicated that the small decay differences due to treatment were not significant. The interaction between treatment and length of storage was not significant in the combined analysis, although it was significant in separate analyses for 2 of the 3 years during which the tests were made. Significantly more decay occurred when the grapes were stored for 5 months than when they were stored for 3 months, irrespective of the frequency of fumigation during storage.

The relation between early or late harvest and frequency of fumigation during storage for the 3-year period is shown in Figure 1, C. Significantly more decay occurred in late- than in early-harvested fruit irrespective of treatment during storage. The interaction between treatment and harvest was not significant.

In the analysis of variance of the combined data for the 3-year period, the interaction between harvest and length of storage was highly significant (LSD = 1.4 per cent at the 5 per cent level) (Fig. 1, A). When the fruit was harvested early in the season there was no significant difference in decay in that stored 3 months or 5 months; however, in late-harvested fruit significantly more decay occurred in fruit stored 5 months than in fruit stored only 3 months. This was also true in 2 of the 3 separate analyses for individual years.

Sulfur dioxide injury.—In the 3 years during which these studies were conducted, no consistent relation between frequency of fumigation during storage and SO_2 injury was found. In the 1952 test, significantly more injury was found in grapes that received the two 1 per cent fumigation treatments (19.8 per cent) than in those that received only a single 1 per cent treatment (15.8 per cent) at the beginning of storage. The double 1 per cent gassing treatment caused more injury in early- than in late-harvested grapes. It was found also in 1951 and 1952 that more injury occurred in early-harvested than in late-harvested grapes irrespective of treatment.

DISCUSSION.—One would expect that more injury would occur in grapes stored for long periods than in those stored for shorter periods; however, aging of the fruit often masked the symptoms of SO_2 injury in late-stored grapes, making a rating for injury very difficult.

Early-harvested grapes had less decay than late-harvested ones. The relation of harvest date and decay has been reported in more detail in connection with forecasting decay in stored grapes (9, 11). A positive correlation between wastage and maturity of grapes has been demonstrated by Rattray (17, 18, 19), duPlessis (8), and Nelson (13). In 2 of the 3 years during which the tests were made, more SO_2 injury was found in early- than in late-harvested grapes. This effect is in accord with the earlier findings of Winkler and Jacob (25), who reported that immature grapes absorb more SO_2 than do mature ones.

The finding that late-harvested grapes stored 3 months had more decay than did early-harvested grapes stored 5 months (Fig. 1, A) suggests that late-harvested grapes should not be stored for long periods, but should be marketed soon after harvest. The effect of harvest date, however, varies greatly with the particular season since field infections that occur under adverse weather conditions are responsible for much of the decay that develops in storage (6, 7, 9, 10, 11, 20).

In numerous instances, fumigation injury appeared to be associated more closely with mechanical injuries at the capstem attachment than with the particular fumigation treatment. The gas penetrates the fruit more readily where the skin is broken. The additional treatment in the 1952 test in which 1 lot of grapes received two 1 per cent SO_2 fumigations indicated that this treatment caused significantly more injury to grapes than the single initial exposure. Preliminary studies on the effect of varying the initial concentration of gas used on grapes indicate that there is a positive correlation between injury and the concentration of gas used in the initial fumigation. Earlier studies on Tokay grapes by Pentzer, Bratley, and Tufts (16) indicated a relation between initial gas concentration and injury. Lowering the concentration of gas, however, also decreases its effectiveness in controlling decay. Studies on the initial sulfur dioxide fumigation are still in progress and will be reported upon at a later date.

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LITERATURE CITED

1. ALLEN, F. W., AND W. T. PENTZER. 1950. (Cold storage of) apples, pears, and grapes. In The refrigerating data book. Amer. Soc. of Refrig. Engin. New York. p. 173-187.
2. ASBURY, C. E., C. O. BRATLEY, AND W. T. PENTZER. 1936. Further observations on mold control in grapes during transit and storage—1935 season. Blue Anchor **13**(6): 8-9, 21.
3. COCHRAN, W. G., AND GERTRUDE M. COX. 1950. Experimental designs. Wiley. New York. 454 p.
4. DELP, C. J., W. B. HEWITT, AND K. E. NELSON. 1951. Cladosporium rot of grapes in storage. Phytopathology **41**: 937-938.
5. DUFRENOY, J., AND L. GENEVOIS. 1935. Développement du Cladosporium herbarum sur des raisins a basse température. Soc. de Biol. [Paris] Compt. Rend.

118: 708-710.
6. DuPlessis, S. J. 1935. Studies on the wastage of grapes. Stellenbosch Univ. Farmers' Bul. 94, 3 p.
7. DuPlessis, S. J. 1936. Studies on the wastage of export grapes with special reference to that caused by Botrytis cinerea, Pers. Union of S. Africa Dept. Agr. Sci. Bul. 151, 163 p.
8. DuPlessis, S. J. 1937. Studies on the physiology and parasitism of Botrytis cinerea Pers. Ann. Appl. Biol. **24:** 733-746.
9. Harvey, J. M. 1954. Forecasting decay in stored California table grapes. Phytopathology **44:** 492.
10. Harvey, J. M. 1955. Decay in stored grapes reduced by field applications of fungicides. Phytopathology **45:** 137-140.
11. Harvey, J. M. 1955. A method of forecasting decay in California storage grapes. Phytopathology **45:** 229-232.
12. Jacob, H. E. 1929. The use of sulfur dioxide in shipping grapes. Calif. Agr. Exp. Sta. Bul. 471, 24 p.
13. Nelson, K. E. 1951. Factors influencing the infection of table grapes by Botrytis cinerea (Pers.). Phytopathology **41:** 319-326.
14. Pentzer, W. T., and C. E. Asbury. 1934. Sulfur dioxide as an aid in the preservation of grapes in transit and storage. Blue Anchor **11**(8): 2-4.
15. Pentzer, W. T., C. E. Asbury, and K. C. Hamner. 1932. Effects of fumigation of different varieties of Vinifera grapes with sulfur dioxide gas. Amer. Soc. Hort. Sci. Proc. **29:** 339-344.
16. Pentzer, W. T., C. O. Bratley, and W. G. Tufts. 1942. Report on sulfur dioxide injury in commercial shipments of Tokay grapes—1942 season. U. S. Hort. Field Sta., Fresno, Calif. Mimeographed Rpt. 11 p.
17. Rattray, J. M. 1935-1936. Grape wastage investigations. Union So. Africa Dept. Agr. and Forestry, Rpt. Low Temp. Res. Lab., Capetown. p. 167-187.
18. Rattray, J. M. 1936-1937. Grape wastage investigations, 1937. Maturity in relation to wastage in Gros Colman from Constantia. Union So. Africa Dept. Agr. and Forestry, Rpt. Low Temp. Res. Lab., Capetown. p. 75-91.
19. Rattray, J. M. 1937-1938. Grape wastage investigations, 1938. Union So. Africa Dept. Agr. and Forestry, Rpt. Low Temp. Res. Lab., Capetown. p. 65-74.
20. Reyneke, J., and S. J. duPlessis. 1939. The cause and control of wastage in grapes. Stellenbosch Univ. Farmer's Bul. 140, 4 p.
21. Rose, D. H., C. O. Bratley, and W. T. Pentzer. 1939. Market diseases of fruits and vegetables: Grapes and other small fruits. U. S. Dept. Agr. Misc. Publ. 340, 27 p.
22. Rose, D. H., and W. T. Pentzer. 1947. Commercial method of fumigating grapes with sulfur dioxide. Blue Anchor. **24**(4): 6-7, 34.
23. Snedecor, G. W. 1946. Statistical methods applied to experiments in agriculture and biology. Collegiate Press, Ames, Iowa. 485 p.
24. Tervet, I. W., A. J. Rawson, E. Cherry, and R. B. Saxon. 1951. A method of collecting microscopic particles. Phytopathology **41:** 282-285.
25. Winkler, A. J., and H. E. Jacob. 1925. The utilization of sulfur dioxide in the marketing of grapes. Hilgardia **1:** 107-131.

PHYTOPATHOLOGICAL NOTES

Nonidentity of Apple Mosaic and Tobacco Streak Viruses. Robert W. Fulton. Yarwood[1] recently described a virus transmitted mechanically to tobacco and certain other plants from an apple tree showing mosaic. He identified the virus as the cause of apple mosaic by transferring it from tobacco to apple through dodder. On the basis of symptoms, host range, and properties, Yarwood suggested that the apple mosaic virus might be a strain of tobacco streak virus.

In order to test this hypothesis by means of a cross-protection test, tobacco leaves infected with apple mosaic virus were obtained through the courtesy of Dr. Yarwood. This virus produced symptoms on tobacco very similar to those of tobacco streak. Lesions on inoculated leaves were somewhat milder than those incited by the common strain of tobacco streak virus, but were within the range of variation shown by different strains of the streak virus. The systemic symptoms were very similar.

When symptomless upper leaves of tobacco (*Nicotiana tabacum* L. vars. Havana 38 and T. I. 787) "recovered" from tobacco streak were inoculated with apple mosaic, however, local and systemic symptoms were produced as on control plants. Similarly, leaves on tobacco "recovered" from apple mosaic virus were not protected against subsequent infection with tobacco streak virus.

Attempts were made to transmit tobacco streak virus from tobacco to apple seedlings through *Cuscuta campestris* Yunck. No infection was obtained in several trials.

On the basis of the lack of cross protection, apple mosaic virus cannot be considered a strain of tobacco streak virus. Similarities, however, in host ranges and properties of these viruses, as pointed out by Yarwood,[1] suggest that they both might be considered members of a larger taxonomic group.—Department of Plant Pathology, University of Wisconsin, Madison, Wisconsin.

[1] Yarwood, C. E. 1955. Mechanical transmission of an apple mosaic virus. Hilgardia 23: 613-628.

Hot-water Treatment of Fungi Infecting Strawberry Roots. P. M. Miller and E. M. Stoddard. Hot-water treatment for 2 minutes at 127° F was found by Goheen and McGrew[1] to kill endoparasitic nematodes

[1] Goheen, A. C., and J. R. McGrew. 1954. Control of endoparasitic root nematodes in strawberry propagation stocks by hot water treatments. U. S. Dept. of Agr. Pl. Dis. Rptr. 38: 818-826.

TABLE 1.—*Length of exposure to hot-water treatment required to kill 3 fungi infecting strawberry roots*

Fungus, age and stage of growth, and medium	Number of minutes[a] at indicated temperature (°F) required to kill							
	105°	115°	122°	125°	127°	131°	140°	149°
R. solani								
2 Days—young mycelium								
On stem sections[b]	>7	1	1	1	1	1	1	1
On agar slants	>7	>7	7	1	1	1	1	1
14 Days—sclerotia, mycelium								
On stem sections[b]	>7	>7	>7	>7	4	1	1	1
On agar slants[b]	>7	>7	>7	>7	4	1	1	1
V. albo-atrum								
2 Days—young mycelium								
On agar slants	>7	>7	>7	>7	4	4	4	1
14 Days—microsclerotia and mycelium								
On agar slants[b]	>7	>7	>7	>7	>7	4(?)[c]	?	?
Fusarium sp.								
2 Days—young mycelium								
On agar slants	>7	>7	>7	>7	>7	>7	>7	7
14 Days—chlamyda-spores, mycelium								
On agar slants[b]	>7	>7	>7	>7	>7	>7	>7	>7

[a] Add 1 minute to each treatment for equalization of temperatures of cultures and water bath. >7 indicates that none of the exposure periods, including the longest (7 minutes), were effective.
[b] Tissues inside tubes were covered with water during heat treatment.
[c] Results were variable, but 4 minutes at 131° F was minimum effective treatment in any test.

in the roots of dormant strawberry runner plants with little or no injury to the plants. This treatment would have added value if it were possible also to kill root-infecting fungi with the same technique. To test this possibility, 3 fungi pathogenic on strawberry roots were heated in a hot water bath at various combinations of time and temperature. The most important point was to find a time and temperature combination lethal to the fungi and noninjurious to strawberry roots. An abstract based on this work was published previously.[2]

Fungi used were *Rhizoctonia solani* Kühn and *Fusarium* sp., both isolated from the roots of Connecticut-grown strawberry plants, and a culture of *Verticillium albo-atrum* Reinke & Bert., originally isolated from strawberries by Dr. Stephen Wilhelm.

These fungi were heated at 2 ages: 2 days and 2 weeks. At 2 weeks, *R. solani* and *V. albo-atrum* were producing sclerotia and microsclerotia, respectively. All 3 fungi were growing on potato-dextrose agar slants—5 ml of agar in each 18 × 150-mm test tube—at the time of heating. In addition, *R. solani* was grown on the surface of sterilized tomato stems in culture tubes. All cultures, except the 2-day cultures on agar, were covered with sterile distilled water prior to heating. It was felt that the mycelium of such young cultures would still be in immediate contact with the agar and thus in a heat-conducting medium. Water was added for 3 reasons: 1) to be sure that all fungal tissues were covered with a heat-conducting medium; 2) it was thought that on strawberry roots the fungi would likely be in the later stages of development and that immersion in water would more closely simulate hot-water treatment of strawberry roots; and 3) because older mycelium and sclerotia might well be more heat-resistant and the immersion in water would improve the kill.

The 3 fungi grown and processed as described above were heated for 1, 4, and 7 minutes in a hot-water bath at temperatures from 105° to 149° F, as indicated in Table 1. One minute was added to each treatment to raise the temperature in the culture tube to that of the water bath. After the treatment, the cultures on agar were incubated for a sufficient length of time to determine the point of complete kill. To determine this point, stems infected with *R. solani* were transferred after treatment to potato-dextrose agar for incubation.

Of the 3 fungi tested, only *R. solani* was killed in all stages of growth by temperatures and times noninjurious to strawberry roots (Table 1). *V. albo-atrum* may have been reduced somewhat, but heat-treatment would appear to be of doubtful value. It is at once evident that *Fusarium* sp. (with a minimum of 7 minutes at 149° F required to kill) cannot be killed by heat treatments sub-lethal to strawberry roots.

From these data it can be concluded that dormant strawberry runner plants can be rid of *R. solani* with treatment with water at a temperature of 127° F for 4 minutes. Goheen, McGrew, and Smith[3] found runner plants of many varieties could be given this treatment without injury if dug when dormant, stored for 2 or more weeks, and planted soon after treatment. Treatment for 2 minutes at 127° could be used to reduce the amount of *R. solani* present on roots of even the more susceptible varieties, although it probably

[2] Miller, P. M., and E. M. Stoddard. 1956. Hot-water treatment of fungi from strawberry roots. (Abs.) Phytopathology 46: 21.

[3] Goheen, A. C., J. R. McGrew, and J. B. Smith. 1956. Tolerance of strawberry plants to hot-water therapy. U. S. Dept. Agr. Pl. Dis. Rptr. 40: 446-451.

would not kill the fungus completely.—Department of Plant Pathology and Botany, The Connecticut Agricultural Experiment Station, New Haven, Connecticut.

Smog Damage to Ferns in the Los Angeles Area. RUTH ANN BOBROV GLATER. Damage to plants from smog has been reported since 1947.[1] Studies to date indicate that Los Angeles smog is a complex of liquids, solids, and gases, comprising more than 50 chemical elements and compounds, and producing, among other effects, low visibility, eye irritation, crop damage, excessive rubber cracking, and odor nuisances. Plant damage is considered to be due to certain intermediate peroxidic products resulting from the chemical combination of unsaturated hydrocarbons with ozone in the atmosphere.[2] Certain species of vegetables, ornamentals, and weeds were found to be singularly sensitive to smog.[3] Gross symptoms in the field vary with each crop. Damage manifests itself on the leaves; some of the symptoms previously reported[4] are a water-soaked appearance of the leaf undersurface (dahlia), "silvering" of the lower surface (spinach, petunia), "browning" of either surface (romaine lettuce, some Chrysanthemum varieties), brown-black mottling (tomato, mallow), increase in anthocyanin (table beet), cork formation (table and sugar beet), tan banding (barley, *Poa annua* L.), and longitudinal tan streaking (oat, corn, many grasses). Whatever the gross appearance of smog injury, the histological picture is the same in all sensitive vegetation.[5] Recently, a new symptom was observed in ferns in response to the polluted air of Los Angeles. In the field, as well as under lath, fern leaves showed characteristic markings following a period of high smog intensity. The gross symptoms were quite distinct from those previously reported on other plants. Damage manifested itself as a tan spotting of leaflets, followed by local or general dehydration of the affected areas, ending in necrosis of the entire leaf.

The typical fern leaf or frond is large, pinnately compound, and often much dissected. The cells are large and many armed, forming a network around a tremendous amount of internal air space (Fig. 1, A). Stomata are limited to the lower leaf surface. In most ferns, the palisade mesophyll is lacking, or if present, only poorly developed; the blade is composed of uniform spongy mesophyll.

Approximately 24 hours following the incidence of smog, tan lesions, commonly near the smaller veins, appear on the leaflets (Fig. 1, D). At this time, damage to the cells directly above the stomata can be seen with a microscope (Fig. 1, B). Since the spongy mesophyll is a very loose tissue, the smog-laden air easily penetrates through the entire leaf (Fig. 1, C). Within 48 hours, part or all of a damaged frond may become completely dehydrated, turn brown and brittle, and crumble on pressure. No pattern of damage such as described previously for other plants[3] is apparent in ferns. An entire frond or isolated portions of individual fronds on a given plant may be damaged. Old, middle-aged, and young leaves appear to be equally sensitive. The growing tip of the plant, with its spirally uncoiling developing leaves (circinate vernation), usually is not damaged. Occasionally, following a long and severe smog period, a young plant may be completely killed, growing point and all. More commonly the plant will continue growth, albeit slowly. Stems, roots, and all vascular elements of the plant remain uninjured.

The general response of the cells of fern leaves to smog is similar to that described in other plants,[6] but differs in 2 ways: 1) in the random distribution of damage markings as contrasted with the restricted smog damage pattern observed in other plants and 2) in the extreme sensitivity of the plant, a condition that results in complete necrosis of many leaves (Fig. 1, E). The specific expressions of damage in ferns may

[1] Middleton, J. T., J. B. Kendrick, Jr., and H. W. Schwalm. 1950. Injury to herbaceous plants by smog or air polution. U. S. Dept. Agr. Pl. Dis. Rptr. 34: 245-252.

[2] Haagen-Smit, A. J., E. F. Darley, M. Zaitlin, H. Hull, and W. Noble. 1952. Investigation on injury to plants from air pollution in the Los Angeles area. Plant Physiol. 27: 18-34.

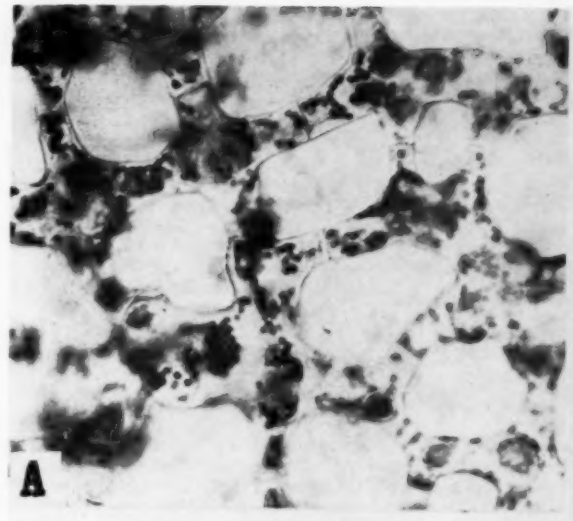
[3] Bobrov, Ruth A. 1955. The leaf structure of Poa annua L. with observations on its smog sensitivity in Los Angeles County. Amer. Jour. Bot. 42: 467-474.

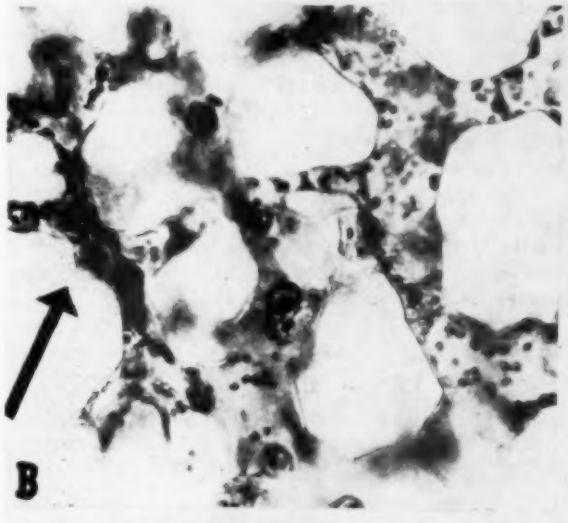
[4] Bobrov, Ruth A. 1952. The anatomical effects of air pollution on plants. 2nd Natl. Air Pollution Symposium Proc. p. 129-134.

[5] Bobrov, Ruth A. 1955. Use of plants as biological indicators of smog in the air of Los Angeles County. Science 121: 510-511.

[6] Bobrov, Ruth A. 1952. The effect of smog on the anatomy of oat leaves. Phytopathology 42: 558-563.

FIG. 1. Smog damage in ferns. A) Paradermal section of normal leaf of *Microlepia platyphylla* J. Smith. Note large, many-armed cells forming network around a tremendous amount of internal air space. B) Paradermal section through early damage. Arrow points to collapsing cells directly above stoma. C) Late damage in cross section. Damage started initially to cells surrounding substomatal chamber. All dark cells are smog damaged; entire lamina would have collapsed soon. Arrow points to stoma on lower surface. D) Early damage manifests itself as tan lesions on leaflets near small veins. E) Late damage in *Lomaria gibba* Labill. Arrows point to completely dehydrated smog-damaged leaves.


A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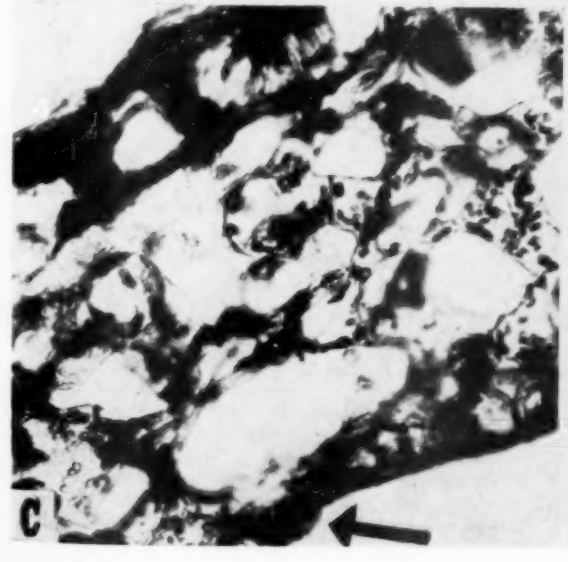
C

D

Lomaria Gibba
E

be attributable to the extreme sponginess of the laminal tissue, through which polluted air can move easily and rapidly, and to the weak suberization of cell walls.[7]—Air Pollution Control District, County of Los Angeles, and Department of Botany, University of California, Los Angeles, California.

Acetylene Microburner for Making Micropipettes for Micromanipulator. A. P. VIEGAS AND E. M. HILDEBRAND. The common laboratory gas used at the Instituto Agronomico, Campinas, was found to be unsuitable for microburners used for making micropipettes for micromanipulators. Apparently the gas has insufficient carbon to be oxidized and thus is unable to reach the temperature required for melting soft glass tubes to obtain the fine point of the micropipette. The difficulty was avoided by using acetylene gas as source of energy for the purpose.

A common 2-piece acetylene generator, of the type used by fishermen in Brazil, was used as source of the acetylene gas (Fig. 1). The lamp was removed, and a rubber tube of convenient diameter was fixed to the exit gas tube. At the end of the rubber tube, an L made of 3-mm glass tubing (Pyrex glass) was inserted. The projecting vertical point of the L was stretched to the desired diameter to produce a pin-sized flame. The actual size of the flame can be regulated by means of a Hoffmann clamp placed on the rubber tube near the base of the microburner proper. This clamp holds the microburner in its vertical position. The vertical portion of the microburner may be of any height convenient for the fabrication of the very fine point of the micropipette.

The writers have never measured the temperature reached at the tip of the pin-sized head of the acetylene flame, but it is high enough to melt glass tubes drawn to the diameter commonly employed in the making of micropipettes. The diameter of the tip of a micropipette is regulated by the tension instantly applied when drawing the glass at the moment it begins to melt.

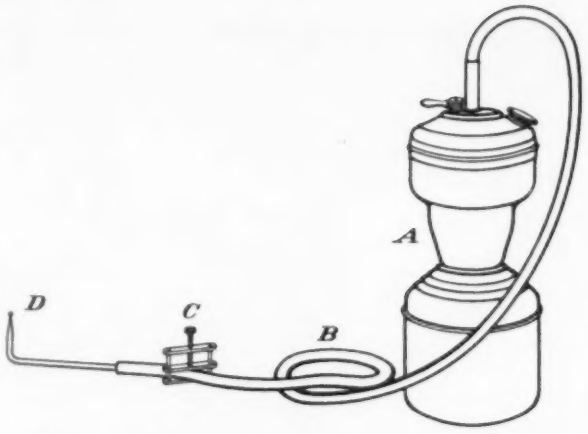

FIG. 1. Acetylene microburner for making micropipettes for micromanipulator. A) 2-piece acetylene generator; B) rubber tubing; C) Hoffmann clamp; D) microflame.

This apparatus probably is excelled by special equipment such as the microforge of de Fonbrune[1,2], but it serves the purpose well where better gas or special apparatus is not available.—Instituto Agronomico, Sao Paulo, Brazil.

[7] Scott, Flora M. 1950. Internal suberization of tissues. Bot. Gaz. 111: 378-394.

[1] De Fonbrune, P. 1937. Micromanipulateur pneumatique et microforge pour la fabrication des micro-instruments. Soc. Indust. Impr., Inst. Pasteur, December, p. 3-22.

[2] De Fonbrune, P. 1939. New methods of micromanipulation. Watson's Micro. Rec. 47, p. 3-8. (Abs. in Roy. Micros. Soc. Jour. III. 59: 320-321. 1939).

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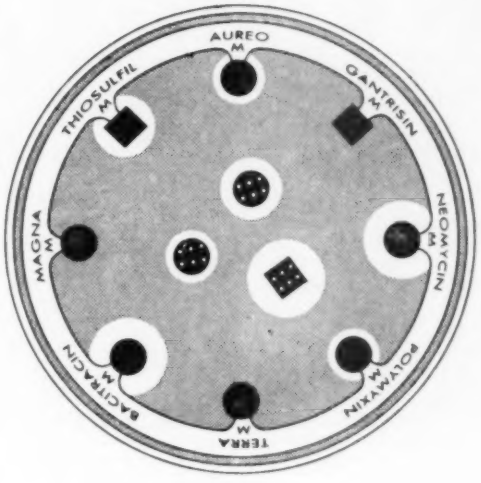

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NEWS

The Department of Botany and Plant Pathology of the Alabama Polytechnic Institute, Auburn, Alabama is now offering the doctoral degree with especial emphasis in plant nematology and herbicidal and seed physiology.

A Committee on standardization of laboratory techniques in soil microbiology was formed at the Technical Committee Meeting of the southern regional project on soil microbiology and plant diseases, held at Knoxville, Tennessee November 5-6. Those in attendance included: Coyt Wilson and E. A. Curl, Alabama Polytechnic Institute; G. M. Armstrong and John H. Bond, Clemson College; J. O. Andes and L. F. Johnson, University of Tennessee; Lyle Koch and Z. A. Patrick, Science Service Laboratory, Harrow, Ontario; W. D. Willis, Louisiana State University, and W. D. McClellan, Washington, D. C.

Plant Pathologist Robert Minoru Takeshita, of the Cereal Crops Section of the U. S. Department of Agriculture, has changed his last name to Endo, effective October 22, 1956. Hereafter, his name will be Robert Minoru Endo.

Phytopathologist John F. Fulkerson has joined the staff of the Forage and Range Section, U.S.D.A. at Beltsville, Maryland. Dr. Fulkerson is a recent graduate of North Carolina State College.

A two-year research grant has been awarded to Josiah L. Lowe by the National Science Foundation. Dr. Lowe, professor of forest botany at the State University College of Forestry at Syracuse University will conduct his research on "A taxonomic study of the Polyporaceae of North America". The research data will be compiled and published in a projected manual on the pore fungi of North America. Dr. Lowe expects to be working on the manual for the next eight or nine years.

The Potomac Division will hold its annual meeting February 28 and March 1, 1957 at Beltsville and Fort Detrick, Frederick, Maryland. Research papers will be presented on that portion of the program held at Beltsville on February 28. On the morning of March 1 the program will be held at nearby Fort Detrick, Frederick, Maryland and will consist of a discussion "Methods and measurements in epiphytology" by members of the staff. The afternoon will be devoted to a tour of facilities and demonstration of equipment.

For sale, if interested write Dr. Walter W. Hansen, Plant Pathology Department, Oklahoma A & M College, Stillwater.

Phytopathology—Vols. 1-6
Phytopathology—Vols. 15-42
Plant Disease Reporter—Vols. 1-25 (mostly complete)
Plant Disease Bulletin supplements from 1919-1941
American Journal of Botany—Vols. 12-25
Science—Vols. 28-110 (mostly complete)

Deaths: Henry L. Bolley, Charter Member of the Society and early researcher in the field of cereal pathology passed away November 9 in Minneapolis, Minnesota at the age of 91. Dr. Bolley was Professor of Botany and Botanist at the North Dakota Agricultural Experiment Station from 1890 until his retirement in 1946. He pioneered in using formaldehyde as a fungicide for controlling seedborne diseases and spent most of his lifetime investigating diseases of flax.

Edwin E. Honey recently retired from Extension Plant Pathology, Pennsylvania State University, passed away October 31 at the age of 65 following a long illness. Dr. Honey received his Ph.D. degree from the University of Wisconsin in 1928. He served as plant pathologist in New York, Illinois, Wisconsin, Washington and in Brazil. Dr. Honey had been on the staff at Penn State since 1948.

Editor: K. W. Kreitlow, Plant Industry Station, Beltsville, Md.

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The *Contents* of these packages prove the value of *Modern Scientific Research*

We are bringing to a close, for the present at least, our program of informational advertisements addressed to the growers of Flue-cured tobacco. It has been our aim in this five-year program to supplement the splendid services of federal and state workers in keeping growers advised of the latest information on tobacco production matters. It has been a stimulating experience for us, and we hope it has been beneficial to you.

The question may be asked, "Why did we undertake this project?" Hanging in offices throughout our organization are brass plaques saying, "Quality of Product is Essential to Continuing Success". That goes for every product we manufacture, and necessarily for the tobaccos we purchase from the growers. Our insistence on quality is one reason why we undertook this program.

In a larger sense, The American Tobacco Company holds to the conviction that a partnership — a community of interest—exists between those who produce leaf tobacco and those who process it into the various forms in which it reaches the consumers. With this thought in mind, we have endeavored to aid the grower in the production of finer tobacco, in more effectively combatting the diseases and insects that attack tobacco, and in other ways fostering economies in his production.

If these advertisements have been useful in that direction, we shall feel that the effort was worthwhile. Moreover, in pursuance of that philosophy, we shall be in readiness to issue supplementary advertisements at such times in the future as changing circumstances may suggest.

In this series, we have never mentioned the brands of cigarettes manufactured by The American Tobacco Company. On this page we picture those brands—the end results of sound production practices which give the American people the best Flue-cured tobacco in the world.

In closing, we wish to express our gratitude to all those federal and state scientists and specialists in tobacco, whose valued counsel and generous assistance in the preparation of these advertisements have made the program possible.

The American Tobacco Company
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TRITHION

(Trade Mark)

(Formerly COMPOUND R-1303)

INSECTICIDE-ACARICIDE

Trithion is a non-systemic, relatively long residual pesticide. It is effective for control of a wide range of insect and mite species. It is active against eggs of various species of mites, including seasonal forms. Its chemical stability and low vapor pressure are sufficient to maintain good residual activity for a moderate period.

Availability: Trithion is suggested for field evaluation in both spray and dust applications. Samples are available for experimental purposes as a liquid concentrate containing 4 pounds of technical Trithion per gallon, a wettable powder containing 25% by weight; and as dusts in 1 to 5% concentrations.

Information: Additional technical information may be obtained from the Agricultural Research Laboratory of the Stauffer Chemical Company, 1496 East Fremont Avenue, Mountain View, California, or Stauffer Chemical Company, Research Center, Chauncey, New York.

Stauffer CHEMICALS SINCE 1885

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